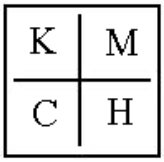
“DEVELOPMENT AND EVALUATION OF COLON TARGETED DRUG DELIVERY FOR TRAMADOL HYDROCHLORIDE”

Dissertation submitted to
The Tamilnadu Dr. M.G.R Medical University, Chennai
In partial fulfillment for the requirement of the degree of

MASTER OF PHARMACY
(Pharmaceutics)

MARCH-2012



DEPARTMENT OF PHARMACEUTICS

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Submitted by

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EVALUATION CERTIFICATE

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DECLARATION

I do hereby declare that this dissertation entitled **"DEVELOPMENT AND EVALUATION OF COLON TARGETED DRUG DELIVERY FOR TRAMADOL HYDROCHLORIDE"** submitted to the Tamil Nadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy in Pharmaceutics** was done by me under the institutional guidance of **Dr.N.ARUNKUMAR M.Pharm., Ph.D.,** Asst. Professor, Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, and Industrial Guidance of **Mr. Mahesh Kamble,** QA Manager NuLife Pharmaceuticals, Pune, during the year 2011 – 2012.

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Dedicated To My

MOM- DAD

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ABBREVIATIONS USED

e.g.	Example
i.e	That is
%	Percentage
kg	Kilogram
mg	Milligram
ml	Milliliters
mm	Millimeters
µg	Microgram
w/w	Weight by weight
w/v	Weight by volume
CoDDS	Colon targeted drug delivery system
PG	Propylene glycol
PEG	Polyethylene glycol
HPMC	Hydroxypropyl methyl cellulose
SASP	Salicylazasulfapyridine (sulfasalazin)
Avg	Average
Hrs	Hours
RPM	Revolution per minute
T	Time
CR	Cumulative release
Abs	Absorbance
Conc	Concentration
Fig	Figure
UV-VIS	Ultraviolet and visible spectroscopy
R^2	Regression coefficient
N	Slope
USP	United state pharmacopoeia
BP	British Pharmacopoeia

ABSTRACT

The present study was aimed to prepare colon targeted tablets containing Tramadol HCl as active pharmaceutical agent and Eudragit E100 and Eudragit S100 as pH dependent polymers and to study the effect of these polymers on drug release rate. Different layers of polymers has been coated on the core tablet by using HPMC as a barrier layer coat to avoid all possible interaction between Eudragit E100 and S100 since they are oppositely charged and different plasticizers PEG 400, Talc and PG were also used to give a proper coating and strength to the tablet. The coating of pH dependent polymers was done at three coating levels to give a net weight increase of about 3, 6 and 9% respectively. The barrier layer coat with HPMC was done to a constant weight increase of about 5%. Thus the core tablets have three layers coating comprising Eudragit S100 as the outer coat, HPMC as the middle layer barrier coat and Eudragit E100 as the innermost coat Evaluation studies were performed which includes all the preformulation and post formulation studies. The core and coated tablets evaluation studies includes hardness, friability, weight variation, drug content, thickness test and weight gain after each successive coating. *In vitro* drug release study was done in USP type II Paddle apparatus in different pH media such as pH1.2 for two hours, pH7.4 for three hours and pH6.8 for nineteen hours to mimic the intestinal pH and transit time. Sampling was done in prescribed time limit and analyzed for drug content using UV spectrophotometer at 271nm wavelength for calculating the drug release.

Formulation F8 with 9% coating level of Eudragit S100 and 6% coating level of Eudragit E100 along with 5% HPMC barrier coating was found to be the optimized one as it showed more than 90% release in the colonic condition, with negligible release in the intestinal pH and it also gave a complete release in 24h. It can be concluded that, the combination of Eudragit E100 and S100 can be used effectively for colon targeting of drugs. Further animal studies for the *in vivo* results are necessary to claim complete success from the study.

Introduction

1. INTRODUCTION

Modified release delivery systems are divided into four, namely, delayed release, sustained release, site-specific targeting and receptor targeting. Colon targeted drug delivery is an example of controlled drug delivery system. The most convenient route of administration of drugs to patients is reputed to be the oral route as against other routes. Upon administration of conventional dosage forms through the oral route they dissolve in the stomach or intestinal fluids from where the absorption of the active drug takes place. The kinetics of absorption of such dosage forms through the mucosal walls depends greatly on the physicochemical properties of the drug in question. Experience shows that conventional drug delivery is unfavorable to special cases where drug targeting is sought, that is, when avoidance of gastric dissolution or targeting to the colon is desirable. Colon targeted drug delivery differs from ordinary enteric coating (that are designed to merely avoid drug release in the stomach) in that the tablet or capsule is specially formulated to channel greater quantity of drug release to the colonic compartment, thus preventing or highly reducing drug release until the dosage form reaches the colon[1] .

Colon has a longer retention time and appears to be highly responsive to agents that enhance the absorption of poorly absorbed drugs. A colon specific drug delivery system is required to protect the drug during its transit through the upper gastrointestinal tract and to allow its release in the colon. It is advantageous if drug release from a formulation can begin immediately after it enters the colon [2].Colon specific drug delivery systems are potential not only for delivering various drugs to combat the local diseases of colon such as crohn's disease, ulcerative colitis, constipation and colon cancer but also for delivering some drugs for the systemic absorption for treating some diseases such as rheumatoid arthritis, nocturnal asthma, hypertension which possess circadian rhythms in their symptoms [3]

Colon specific drug delivery systems (CoDDS) have been developing as one of the site-specific drug delivery systems. Along with many applications in local and systemic delivery of drugs the CoDDS would also be advantageous when a delay in absorption is desirable from a therapeutic point of view as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythm, such as nocturnal asthma, angina and rheumatoid arthritis. So by developing the pulsatile device for specific colonic delivery, plasma peak is

obtained at an optimal time, number of doses per day can be reduced, first pass metabolism and tolerance development can also be avoided [4]

1.1 Rationale for colon targeting [5, 6]:

Colon-specific drug delivery system offers the following therapeutic advantages;

- Reducing the adverse effects in the treatment of colonic diseases (ulcerative colitis, colorectal cancer, crohn's disease etc.)
- By producing the 'friendlier' environment for peptides and proteins when compared to upper gastrointestinal tract.
- Delayed release of drugs to treat early morning attacks of angina, asthma and rheumatoid arthritis
- Reduced incidence of side effects and drug interactions.
- Bypass initial first pass metabolism, extended daytime or nighttime activity.
- Improve patient compliance.
- It has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs

1.2 limitations of colon targeting [7]:

- Location at distal portion of alimentary canal, the colon is difficult to access.
- Successful delivery requires the drug to be in solution before it arrives in the colon, but fluid content in the colon is lower and more viscous than upper GIT, which is the limiting factor for poorly soluble drugs.
- Lower surface area and tightness of light junctions in colon can restrict drug transport across mucosa into systemic circulation.

1.3 Anatomy and physiology of colon [8]:

The GI tract is divided into stomach, small intestine and large intestine. The large intestine extending from the ileocecal junction to the anus is divided in to three main parts. These are the colon, the rectum and anal canal. The entire colon is about 5feet (150cm) long, and is divided in to five major segments. Peritoneal folds called as mesentery which is supported by ascending and descending colon. The right colon consists of the cecum, ascending colon, hepatic flexure and the right half of the transverse colon The left colon contain the left half of the transverse colon, descending colon, splenic flexure and sigmoid. The rectum is the last anatomic segment before the anus.

The major function of the colon is the creation of suitable environment for the growth of colonic microorganisms, storage reservoir of faecal contents, expulsion of the contents of the colon at an appropriate time and absorption of potassium and water from the lumen. The absorptive capacity is very high, about 2000ml of fluid enters the colon through the ileocecal valve from which more than 90% of the fluid is reabsorbed. On average, it has been estimated that colon contains only about 220gm of wet material equivalent to just 35gm of dry matter. The majority of this dry matter is bacteria. The colon tissue contains the villi, lymph, muscle, nerves, and vessels.

1.3.1 Ascending colon [9]:

The ascending colon is approximately 15cm long and joins the caecum at the ileocaecal junction. The ascending colon is covered with peritoneum anteriorly and on both sides, however, its posterior surface is devoid of peritoneum. It ascends on the right side of the abdomen to the level of the liver where it bends acutely to the left. At this point it forms the right colic or hepatic flexure and then continues as the transverse colon.

1.3.2 Transverse colon:

This is a loop of colon approximately 45cm long that continues from the left hepatic flexure across to the left side of the abdomen to the left colic flexure. It passes in front of the stomach and duodenum and then curves beneath the lower part of the spleen on the left side as the left colic or splenic flexure and then passes acutely downward as the descending colon.

1.3.3 Descending colon:

This section of the colon passes downwards on the left side of the abdomen to the level of the iliac crest. It is approximately 25cm in length. The descending colon is narrower and more dorsally situated than the ascending colon.

1.3.4 Sigmoid colon:

The sigmoid colon begins near the iliac crest and is approximately 36cm long. It ends at the centre of the mid-sacrum, where it becomes the rectum at about the level of the third sacral vertebra. It is mobile and is completely covered by peritoneum and attached to the pelvic walls in an inverted V shape.

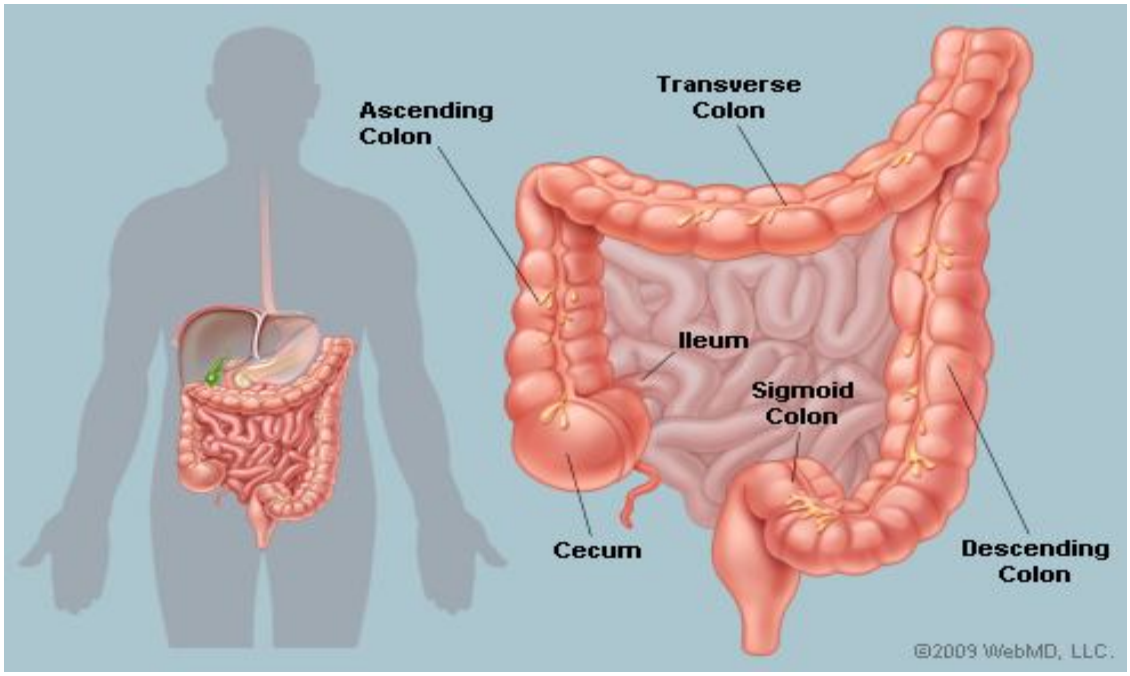


Fig 1: Human Intestinal Colon

Table 1: Summary of anatomical and physiological feature of small intestine and colon [10]:

Region of Gastrointestinal Tract		Length (cm)	pH	Internal diameter (cm)
Stomach		------	1.5-3 (fasted) 2-5 (fed)	-----
Small intestine	Duodenum	20-30	≈6.1(fasted) ≈5.4(fed)	3-4
	Jejunum	150-200	≈5.4	
	Ileum	200-350	≈7-8	
Large intestine	Cecum	6-7	≈ 5.5-7	6
	Ascending colon	20		
	Transverse colon	45		
	Descending colon	30		
	Sigmoid colon	40	7-8	
	Rectum	12		
	Anal canal	3		

The best Candidates for CoDDS are drugs which show poor absorption from the stomach or intestine including peptides. The drugs used in the treatment of IBD, ulcerative colitis, diarrhea, and colon cancer are ideal candidates for local colon delivery. The criteria for selection of drugs for CoDDS is summarized in this table 2

Table 2: Criteria for selection of drug for CoDDS [11]:

CRITERIA	PHARMACOLOGICAL CLASS	NON-PEPTIDE DRUGS	PEPTIDE DRUGS
Drugs used for local effects in colon against GIT diseases	Anti-inflammatory drugs	Oxyprenolol, Metoprolol, Nifedipine	Amylin, Antisense oligonucleotide
Drugs poorly absorbed from upper GIT	Antihypertensive and antianginal drugs	Ibuprofen, Isosorbides, Theophylline	Cyclosporine, Desmopressin
Drugs for colon cancer	Antineoplastic drugs	Pseudoephedrine	Epoetin, Glucagon
Drugs that degrade in stomach and small intestine	Peptides and proteins	Bromophenaramine, 5-Flourouracil, Doxorubicin	Gonadoreline, Insulin, Interferons
Drugs that undergo extensive first pass metabolism	Nitroglycerin and corticosteroids	Bleomycin, Nicotine	Protirelin,sermorelin, Saloatonin
Drugs for targeting	Antiarthritic and antiasthamatic drugs	Prednisolone, hydrocortisone, 5-Amino-salicylic acid	Somatropin,Urotoilitin

1.4 Colonic absorption of drug [7]:

The surface area of colon is compensated by absence of endogenous digestive enzymes and long residence time of colon (10-24hours).transcellular absorption involves the passage of drug through the cells and thus the route for most lipophilic drugs, whereas paracellular absorption involves the transport of drug through the tight junction between the cells and thus the route for most hydrophilic drugs.

1.4.1 Factors affecting colon absorption [12]:

- Passage through coloncytes(transcellular transport)
- Passage through adjacent coloncytes(paracellular transport)
- Physical properties of drug such as pKa and degree of ionization.
- Colonic residence time as commanded by GIT motility.
- Degradation by bacterial enzymes and metabolic products.
- Local physiological action of drug.
- Selective and non-selective binding to mucus.
- Disease state.

1.5 Advantages of CoDDS over conventional drug delivery [10, 13]:

- Chronic colitis, namely ulcerative colitis and cirrhosis disease are currently treated with glucocorticoids, and other anti-inflammatory agents.
- Drugs are available directly at the target site.
- Side effects can be reduced.
- Utilization of drug is more.
- Lesser amount of dose is required comparatively.

1.6 Factors to be considered in the design of colon-specific drug delivery system [14]

1.6.1 Colonic micro flora and their enzymes [14]

Intestinal enzymes are used to trigger drug release in various parts of the GI tract. Usually these enzymes are derived from gut microflora residing in high numbers in the colon. These enzymes are used to degrade coatings/matrices as well as to break bonds between an inert carrier and an active agent (i.e. release of a drug from a prodrug). Over 400 distinct bacterial species have been found, 20% to 30% of which are of the genus Bacteroides. The upper region of the GI tract has a very small number of bacteria and predominantly consists of Gram-positive facultative bacteria. The concentration of bacteria in the human colon is 1011 to 1012 CFU/ml. The most important anaerobic bacteria are Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium, and Clostridium.20 a summary of the most important metabolic reactions carried out by intestinal bacteria is provided in Table.3

Table 3: Metabolic reactions carried out by intestinal bacteria:

Enzymes	Microorganism	Metabolic reaction catalyzed
Nitroreductase	E. coli, Bacteroides	Reduce aromatic and heterocyclic nitro compounds
Azoreductase	Clostridia, Lactobacilli, E. coli	Reductive cleavage of azo compounds
Esterase and amidases	E. coli, P. vulgaris, B. subtilis, B. mycoides	Cleavage of esters or amidases of carboxylic acids
Glycosidase	Clostridia, Eubacterium	Cleavage of β-glycosidase of alcohols and phenols
Glucuronidase	E. coli, A. aerogenes	Cleavage of β-glucuronidases of alcohols and phenols

1.6.2 pH in the Colon[15]:

The average pH of the caecum and colon lumen is 6.8 ± 0.85. The highest pH levels (7.5 ± 0.5) were found in the terminal ileum. On entry into the colon, the pH dropped to 6.4 ± 0.6. The pH in the mid-colon was measured at 6.6 ± 0.8 and in the left colon, 7.0 ± 0.7. The fall in pH on entry into the colon is due to the presence of short chain fatty acids arising from the bacterial fermentation of polysaccharides for example, lactose is fermented by colonic bacteria to produce large amounts of lactic acid, resulting in a drop in the pH to about 5.0. Colonic pH has been shown to be reduced in disease like ulcerative colitis. Diet, diseased state, and food intake influence the pH of the gastrointestinal fluid.

1.6.3 Gastrointestinal transit [16]

The gastric emptying of dosage form is highly variable and depends primarily on whether the subject is fed or fasted and the property of dosages form (such as size and density). The mean transit time from mouth to anus is 53.3hrs. The total mean colonic transit time is 25.0 hrs and is shorter in males than females. The effect of transit abnormalities has been examined using gamma scintigraphy in which oral administration of a radiolabelled liquid meal to patients takes place. Transit time of dosage form in GIT is given in Table-4

Table 4: Transit time of dosage form in GIT

Organ	**Transit time (hr)**
Stomach	<1 (Fasting) >3 (Fed)
Small intestine	3-4
Large intestine	20-30

1.7 Approaches for colonic drug delivery [17]

[A]- Primary approaches for CoDDS

(a) Microbially triggered drug delivery to colon.

(i) Prodrug approach
(ii) Azo bond conjugate
(iii) Polysaccharide based approach

b) Delayed release or Time controlled release system
c) pH sensitive systems

[B]- Newly developed approaches for CoDDS

a) Pressure controlled drug delivery system (PCDDS)
b) Osmotic controlled drug delivery to colon (OROS-CT)
c) CODES^{TM} (a novel colon targeted delivery system)

[A]- Primary approaches for CoDDS

a) Microbially triggered drug delivery to colon [18- 22].

When the dosage form passes through the GIT, it remains intact in the stomach and small Intestine where very little microbial degradable activity is present which is insufficient for cleavage of the polymer coating. The microflora of colon is in the range of 1011 -1012 CFU/mL, consisting mainly of anaerobic bacteria, e.g**.** Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Enterococci, Enterobacteria and Ruminococcus etc. This vast microflora fulfills its energy needs by fermenting various types of substrates that have been left undigested in the small intestine, e.g. di- and tri-saccharides, polysaccharides etc [20]. For this fermentation the microflora produces a vast number of enzymes like glucoronidase, xylosidase, arabinosidase, galactosidase, nitroreductase, azareducatase, deaminase, and urea dehydroxylase. Because of the presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers for colon-specific drug delivery seems to be a more site-specific. These polymers shield the drug from the environments of stomach and small intestine and are able to deliver the drug to the colon. On reaching the colon, they undergo assimilation by micro-organism or degradation by enzyme or break down of the polymer back bone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength.

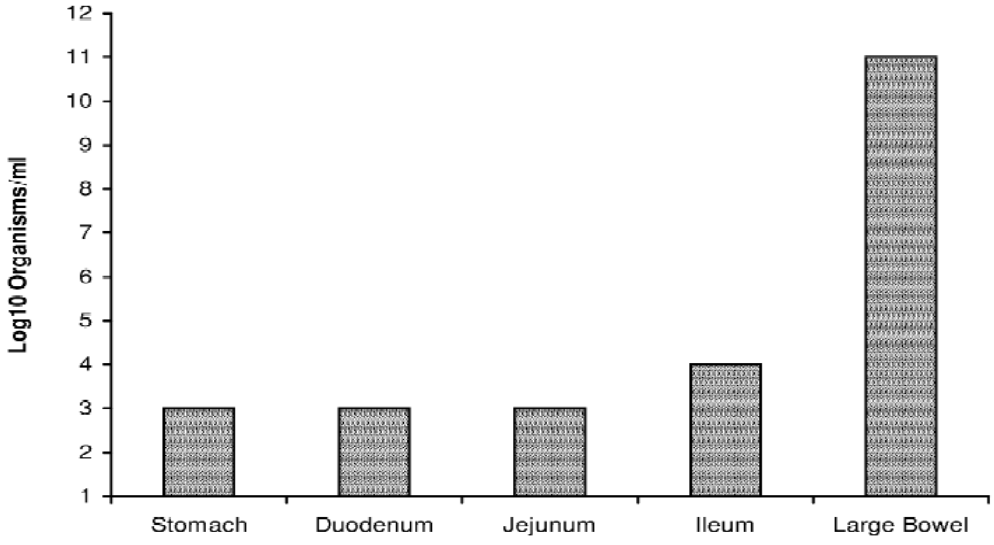


Fig 2: Concentration of bacterial flora in different regions of GIT

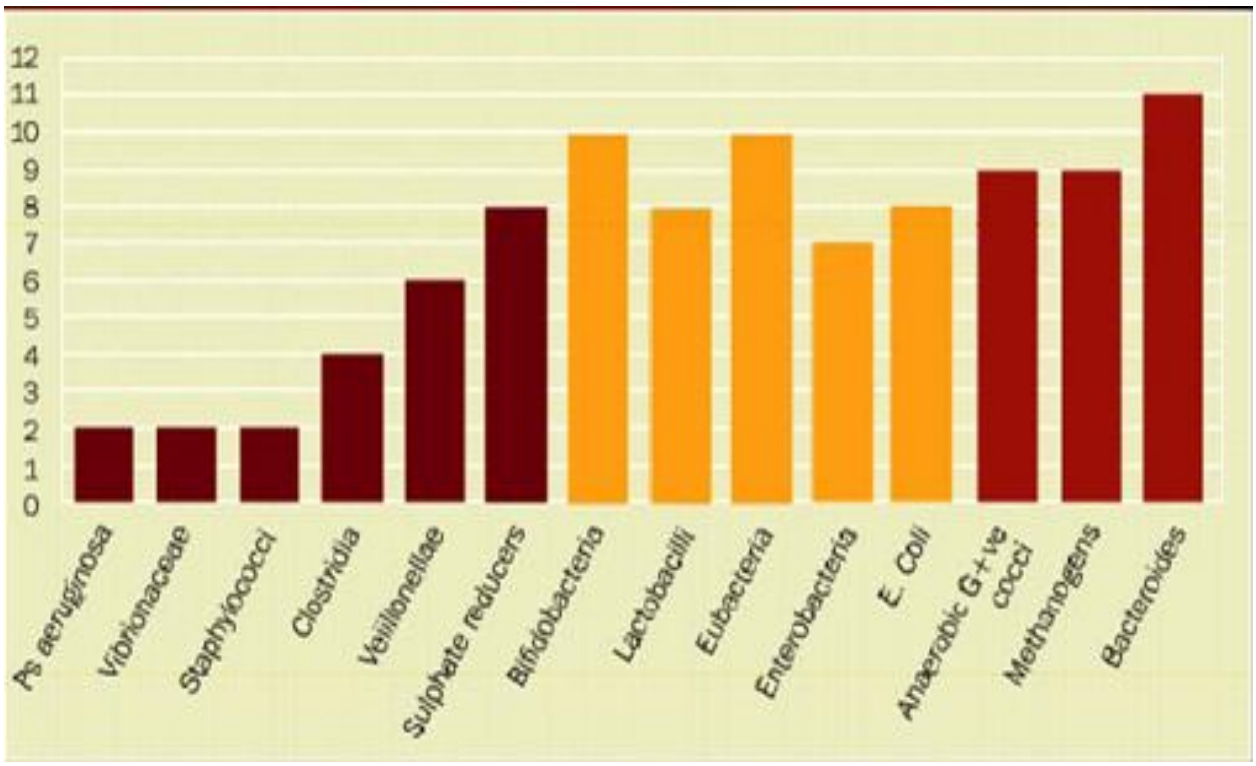


Fig 3: Human intestinal microflora distribution in number (log 10) per gram faeces

(i) Prodrug approach [23, 24]:

Prodrugs have been used in targeting drugs to the colon. Prodrugs are designed to undergo minimal absorption and hydrolysis in the tracts of upper GIT and undergo enzymatic hydrolysis in the colon, there by releasing the active drug moiety from the drug carrier. Subsynthetic polymers have been used to form polymeric prodrug with azo linkage between the polymer and drug moiety. The list of different prodrug is given in table 5.

SASP is the classic colon specific prodrug originally discovered in the late 1930's by the Scandinavian physician Dr. Nanna svartz [26]

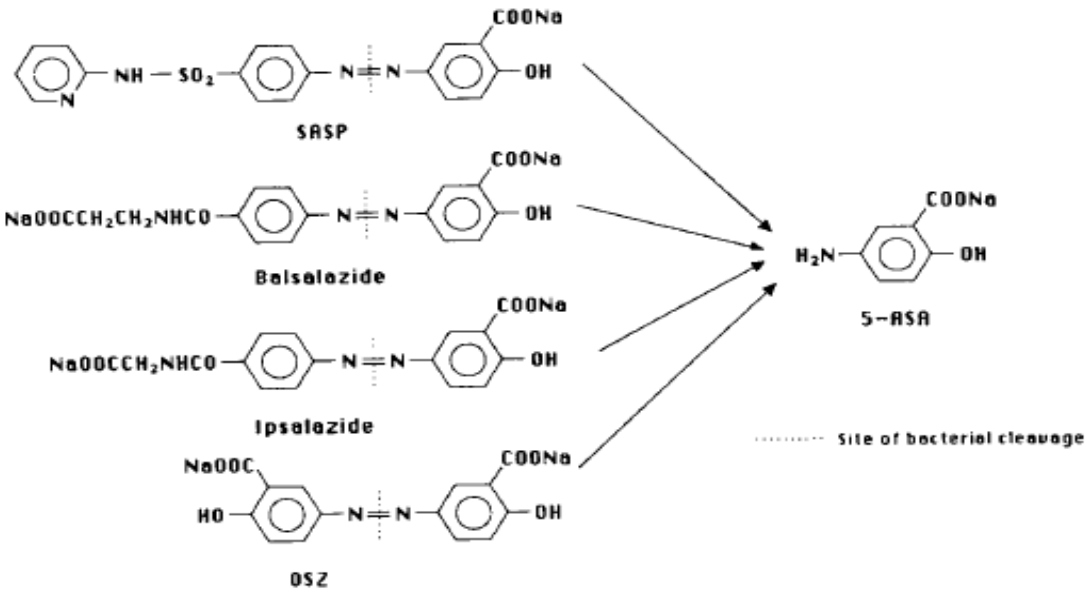


Fig 4: The chemical structure of SASP, Balsalazide, Ipsalazide, and OSZ showing the site of bacterial cleavage leading to formation of active agent 5-asa (SASP-salicylazasulfapyridine (sulfasalazin) OSZ-osalazine)

Table 5: Prodrugs evaluated for colon-specific drug delivery [38]

Drug investigated	**Carrier**	**Linkage hydrolyzed**
5-ASA	Azo conjugates sulphapyridine(SP)	Azo linkage
5-ASA	5-ASA	Azo linkage
Dexamethasone/ Prednisolone	Saccharide carriers	Glycosidic linkage
Dexamethasone; Prednisolone Hydrocortisone,Fludrocortisone	Glucose/galactose/cellobioside	Glycosidic linkage
Nalaxone/Nalamefene	Glucoronide conjugates Glucoronic acid	Glucuronide linkage
Salicylic acid	Amino acid conjugates glycine	Amide linkage

(ii) Azo bond conjugate[18]

These azo compounds are extensively metabolized by the intestinal bacteria, both by intracellular enzymatic component and extracellular reduction. Newer approaches are aimed at use of polymers as drug carriers for drug delivery to the colon. Both synthetic as well as naturally occurring polymers are used for this purpose. Subsynthetic polymers have been used to form polymeric prodrug with azo linkage between the polymer and drug moiety. These have been evaluated for CoDDS; various azo polymers have also been evaluated as coating materials over drug cores. These have been found to be similarly susceptible to cleavage by the azoreductase in the large bowel. Coating of peptide capsules with polymers cross linked with azoaromatic group has been found to protect drug from digestion in the stomach and small intestine. In the colon the azo bonds are reduced and the drug is released.

(iii) Polysaccharides based systems[25]

Polysaccharides are the polymers of monosaccharides which retains their integrity because they are resistant to the digestive action of gastrointestinal enzymes. The matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine; however, they are acted upon by the bacterial polysaccharidases once they reach in the colon resulting in the degradation of the matrices. Natural polysaccharide polymers have an appeal to the area of drug delivery as they are comprised of polymers having a large number of derivatizable groups, a wide range of molecular weights, biodegradability, varying chemical compositions, a low toxicity yet high stability. They are already approved as pharmaceutical excipient. The number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans and locust bean gum has been investigated for their use in colon targeted drug delivery systems. The selection of a suitable biodegradable polysaccharide is the most important factor in the development of polysaccharide derivatives for colon targeted drug delivery.

Table 6: Polysaccharides investigated for colon-specific drug delivery [38]

Polysaccharide investigated	Drug moiety used	Dosage form prepared
Chitosan	Sodium diclofenac	Enteric-coated chitosan microspheres
Amidated pectin	Paracetamol	Matrix tablets
Amidated pectin	Indomethacin Sulphamethoxazole	Chitosan-coated amidated pectin beads
pH-sensitive dextran	Bovine serum albumin (BSA)	As hydrogels
Amidated pectin	Ropivacaine	Matrix tablet
Locust bean gum	Theophylline	Film
Dextran fatty acid esters (Degree of substitution 20.12–0.40)	Theophylline	As films

b) Delayed release or Time controlled release system [27]:

Sustained release dosage forms are designed to prolong drug dissolution and hence absorption. These formulations move down the GIT at rates dependent on their location as drug is released from the formulation as it passes down the gut, it is absorbed at a rate depending on the drug's permeability properties and other factors. Unabsorbed drug or drug not released from the formulation is excreted in the faeces. Although less sophisticated than other approaches to targeting drug release in the GI tract, simple sustained release systems are used to deliver drugs to various sites. Sustained release is the basis of Pentasa R (mesalamine) [27], which relies on ethylcellulose-coated beads to slowly release mesalamine (5-aminosalicylic acid, 5-ASA) as they pass down the GIT. It is indicated for treatment of UC; despite the fact inflammation is located in the distal intestine. The relative bioavailability of mesalamine from this formulation is low. If mesalamine is released from the formulation but unabsorbed, it can still reach the inflamed mucosa and possibly exert a local anti-inflammatory effect. Following gastric emptying, transit through the small bowel is relatively consistent at 3±1 h. Thus, it is possible to exploit small intestinal transit times to control the site of drug release. Since gastric residence times are often

variable and dependent on the presence or absence of food, enteric polymers are used to delay release until the formulation reaches the proximal small intestine. Thus, a dual mechanism is used to delay release until the dosage form reaches the lower intestine.

c) pH sensitive system[7,40,41]:

pH dependent coatings make possible the design of dosage forms containing high levels of drugs, as alternatives to matrix or hydrogel systems: using polymers dissolving at pH> 7 for e.g. Eudragit [(41)] it is possible to prevent tablets or pellets from releasing drugs in the stomach or proximal small intestine in stomach pH ranges between 1-2during fasting but increases after eating. The pH is 6.5 in proximal small intestine and about 7.5 in distal small intestine from ileum to colon pH declines significantly. It is about 6.4 in the caecum. pH values as low as 5.7 have been measured in ascending colon in healthy volunteers. The pH in the transverse colon is 6.6 in descending colon 7.0 use of pH dependent polymers is based on these differences in pH levels. The polymers described as pH dependent in colon specific drug delivery are insoluble at low pH levels but become increasingly soluble as pH rises. Although a pH depend polymer can protect a formulation in stomach and proximal small intestine. It may start to dissolve even in lower small intestine and the site specificity of the formulation can be poor. Enteric coated dosage forms are designed to remain intact in the stomach and release the active substances in intestine. pH sensitive coating can be used to deliver the drug to the colon. Unit dosage forms and multiparticulate dosage forms have been coated with pH dependent polymers to provide site specific release.

[B]- Newly developed approaches for CoDDS

(a) Pressure controlled drug delivery system (PCDDS) [28, 39]:

The digestive processes within the GI tract involve contractile activity of the stomach and peristaltic movements for propulsion of intestinal contents. In the large intestine, the contents are moved from one part to the next, as from the ascending to the transverse colon by forcible peristaltic movements commonly termed as mass peristalsis. These strong peristaltic waves in the colon are of short duration, occurring only three to four times a day. However, they

temporarily increase the luminal pressure within the colon, which forms the basis for design of pressure-controlled systems. The use of gastrointestinal pressure has been proposed as a method of targeting release in the distal gut. This pressure which is generated via muscular contractions of the intestinal wall for grinding and propulsion of luminal contents varies in intensity and duration throughout the gastrointestinal tract. The colon is believed to exert a higher effective luminal pressure via the action of haustral contractions coupled with a viscous environment. This pressure controlled colon delivery capsule (PCDC) is composed of drug, dispersed in a suppository base, coated with the water insoluble polymer ethyl cellulose. Once swallowed, the temperature of the body causes the suppository base to melt and increase in volume, and the system resembles a liquid filled ethyl cellulose balloon. The system is able to withstand the luminal pressures of the stomach and small intestine resulting from muscular con- traction of the gut wall, since there is sufficient fluid present in the lumen to dissipate this pressure. In the distal gut, reabsorption of water increases the viscosity of luminal contents. As such, the capsule will be directly affected when subjected to the pressure of the intense haustral contractions of the colon and hence will rupture. The use of gastrointestinal pressure provides an innovative approach to targeting drugs to the gut.

(b) Osmotic controlled drug delivery to colon (OROS-CT) [29,43]

Linkwitz et al [42] invented an osmotic drug (single unit) delivery capsule from which the delivery of the drug was driven by the osmotic infusion of the moisture by the capsule from a physiological environment. It was provided with a delivery orifice that opened intermittently to achieve a pulsatile delivery effect. The technology involves a movable position that divides the capsule interior into two compartments- one for the beneficial agent and the other for the osmotically active agent. The orifice is located on the capsule wall surrounding the beneficial agent side. The whole capsule is surrounded by an elastic wall in the osmotically active compartment due to inward diffusion of water, which is transmitted through the partition. When the pressure in the drug compartment exceeds the threshold, it results in opening of the orifice and as a result, an amount of beneficial agent is released and it relieves the pressure in the drug compartment. This release in pressure causes the elastic material to relax and results in closures of the orifice. There are various factors affecting the degree and manner in which the pulsatile effect can be controlled, like the choice of elastic material, the thickness of the wall section, the

configuration and location of the orifice and the viscosity and surface tension of the beneficial agent formulation. The choice of elastic material is done on the ability to stretch at least twice their original length and to retract very rapidly when released.

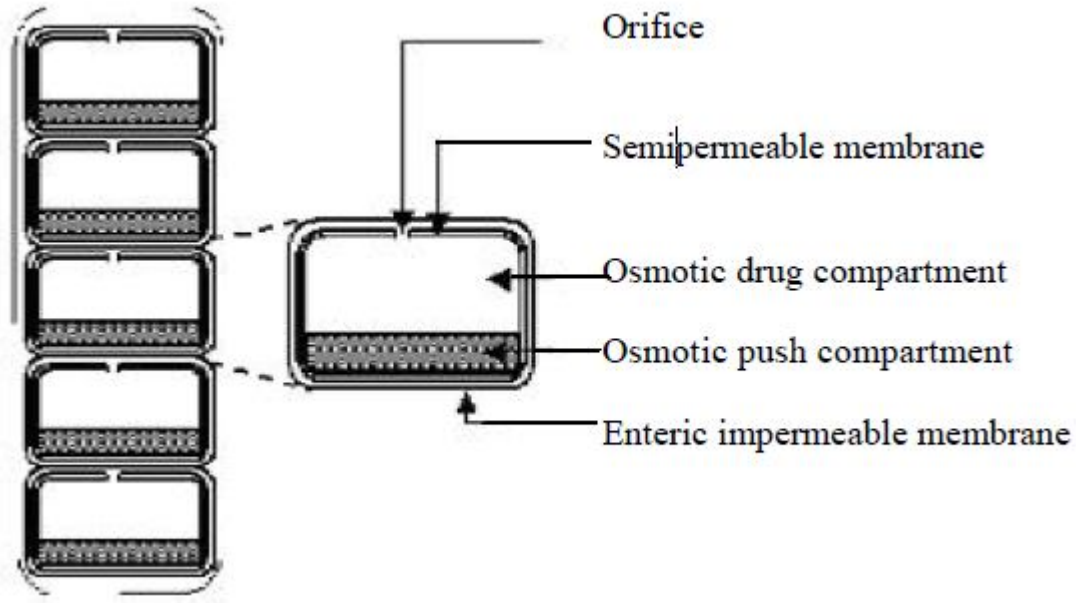


Fig 5: Osmotic drug delivery system

(c) CODES™ (a novel colon targeted delivery system) [29, 30]:

CODES™ is a unique colon-specific drug delivery technology that was designed to avoid the inherent problems associated with pH- or time-dependent systems. The design of CODES™ exploited the advantages of certain polysaccharides that are only degraded by bacteria available in the colon. This is coupled with a pH-sensitive polymer coating. Since the degradation of polysaccharides occurred only in the colon, this system exhibited the capability to achieve colon delivery consistently and reliably. As schematically presented in Fig.5 typical configuration of CODES™ consists of a core tablet coated with three layers of polymer coatings. The first coating (next to the core tablet) is an acid-soluble polymer (in the present case, Eudragit E was used) and outer coating is enteric with a HPMC barrier layer in between to prevent any possible interactions between the oppositely charged polymers.The core tablet is comprised of the active, one or more polysaccharides and other desirable excipients. The polysaccharides, degradable by

enterobacteria to generate organic acid, include mannitol, maltose, stachyose, lactulose, fructooligosaccharide etc. During its transit through the GI tract, CODES™ remains intact in the stomach due to the enteric protection, but the enteric and barrier coating will dissolve in the small intestine, where the pH is above 6. Because Eudragit E starts to dissolve at pH5, the inner Eudragit E coating is only slightly permeable and swellable in small intestine. Upon entry into the colon, the polysaccharide inside the core tablet will dissolve and diffuse through the coating. The bacteria will enzymatically degrade the polysaccharide into organic acid. This lowers the pH surrounding the system sufficient to affect the dissolution of the acid-soluble coating and subsequent drug release.

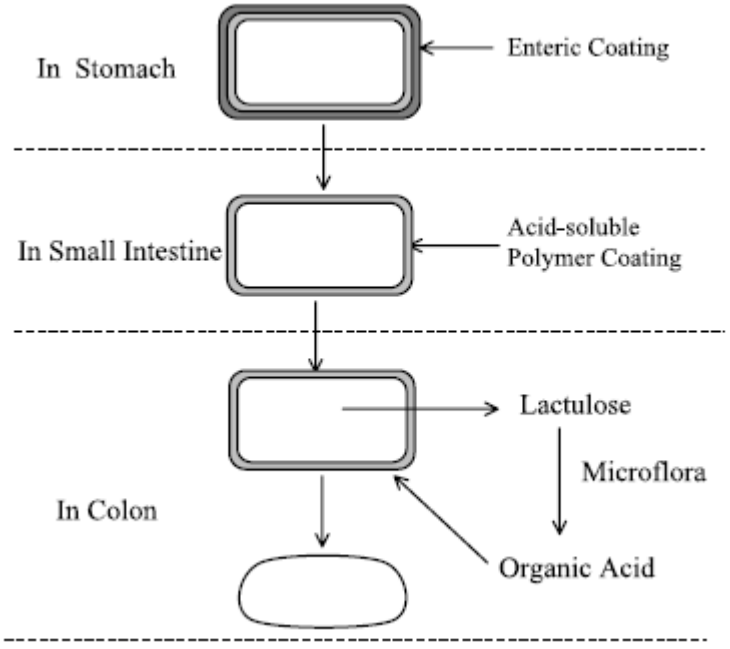


Fig 6: Schematics of the conceptual design of CODES™

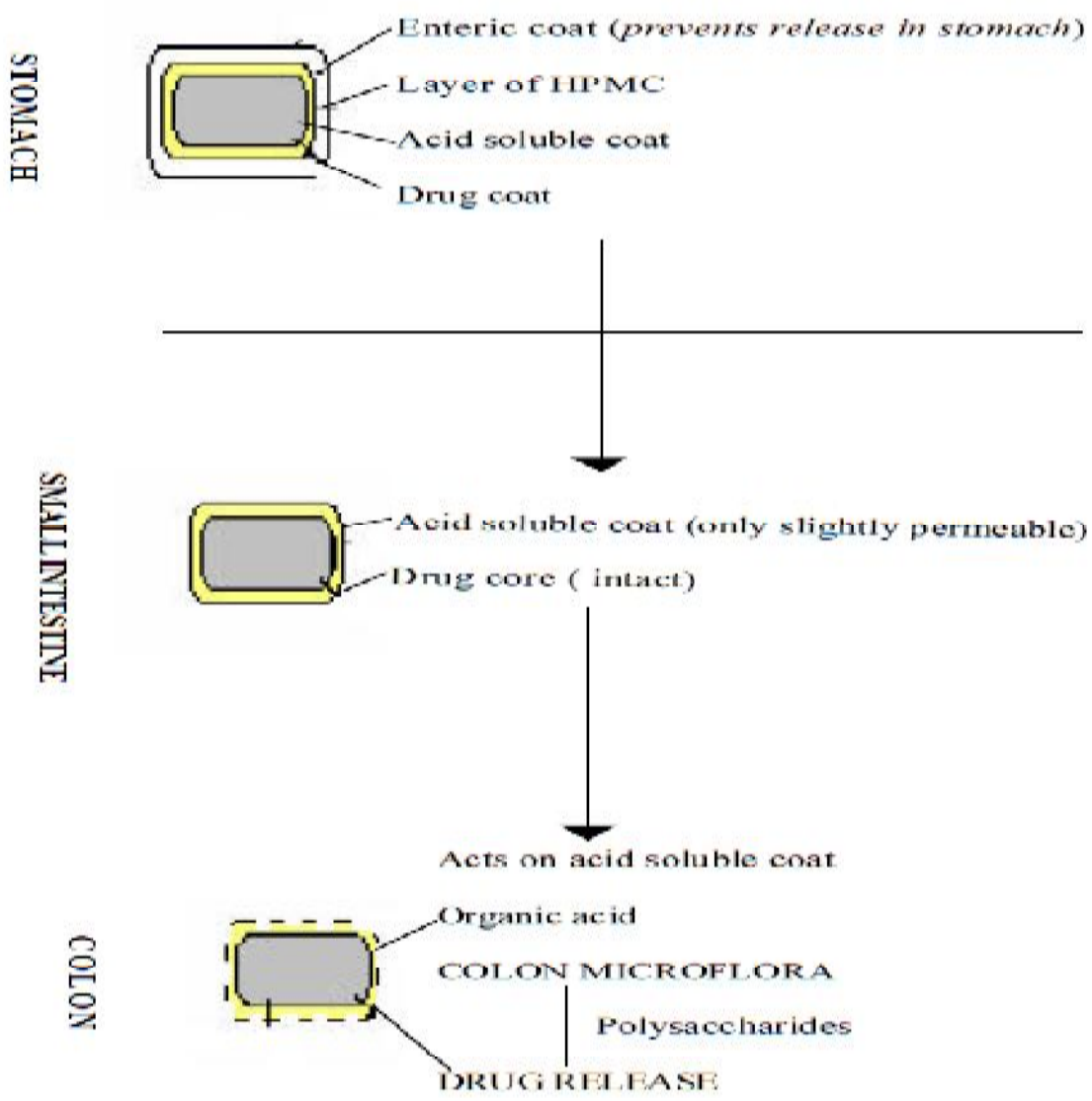


Fig 7: Schematics of the conceptual design of CODES™

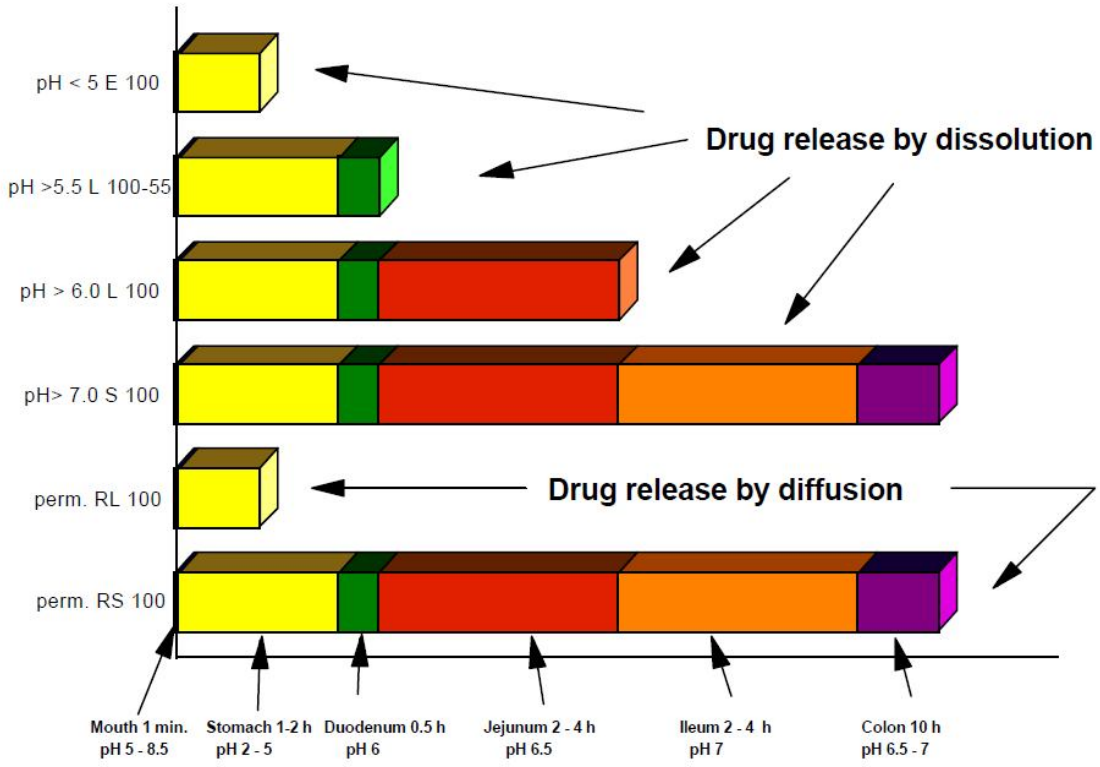


Fig 8: Behavior of Eudragit polymers in digestive tract

The CoDDS can be used as an efficient dosage form to deliver the drugs to the colon for effective treatment of local or systemic disease of colon and for the timed delivery of drugs in case of disease following circadian rhythm.

Plan of Work

OBJECTIVE AND PLAN OF WORK

OBJECTIVE

To formulate and evaluate colon targeted drug delivery system of Tramadol hydrochloride by using different polymers such as Eudragit E100, HPMC, and Eudragit S100.

PLAN OF WORK

- To determine the λ max of the drug and construct the calibration curve
- To conduct the preformulation studies
- To formulate colon targeted drug delivery system
- To carry out physical evaluation tests
- To carry out the *In vitro* dissolution studies
- To determine the effect of formulation parameters on dissolution
- Determination of release kinetics.
- Stability studies.

Literature Review

Patel T.D. et al [52] has investigated the development of delayed release (DR) tablet of Mesalazine, where Lactose-based placebo tablets were coated using methacrylic acid copolymers Eudragit S100 by spraying from aqueous and organic system & Instacoat IEN-II-218 by spraying from organic system. The Dissolution studies performed on the mesalazine tablets demonstrated that a Eudragit S100 can be successfully used for aqueous & organic system to coat tablets for colon targeted delivery of drug. Among the ten formulations**,** F9 best matched formulation with respect to market product. Optimized formulation was found stable during accelerated stability study for 3 months at 40^0C/75% RH. It is concluded that Eudragit S100 far more superior than Instacoat IEN-II-218.

S. J. Kshirsagar et al [53] developed the polymer coated Diclofenac tablet Eudragit FS 3D and Eudragit S100 were used as pH sensitive polymers. Tablets were coated separately with Eudragit FS 30D and Eudragit S100 in various thicknesses and evaluated for *in vitro* drug release using changing pH method. *In vitro* release studies reveals that Eudragit FS30D coated tablet with 10%w/w coating level start release of drug at pH 6.8 after suitable lag time in the same pH which corresponds to colonic arrival time, as compare to Eudragit S100 coated tablet which release only at higher pH , approximating the transverse colon. *In vivo* study shows that Tablet coated with EudragitFS30D with 10%w/w coating level and Eudragit S100 with 10% coating level disintegrated in cecum region. Thus Eudragit FS30D coated tablet may be a promising system for the treatment of colonic disease

Gang Cheng et al [54] has investigated Time- and pH-dependent colon-specific drug delivery systems (CoDDS) for orally administered diclofenac sodium (DS) and 5-aminosalicylic acid (5-ASA), respectively by using Ethylcellulose (EC) and methacrylic acid copolymers (Eudragit L100 and S100), respectively as coating polymer. The *in vitro* release behavior of the DS coated tablets and 5-ASA coated pellets were examined, and then *in vivo* absorption kinetics of DS coated tablets in dogs were further studied. Result was demonstrated that thicker the coating layer, longer the lag time of DS release. The absorption kinetic studies in dogs demonstrated that *in vivo* lag time of absorption was in a good agreement with *in vitro* lag time of release.

Varshosaz J. et al [55] has studied the development of Budesonide pellets based on colon drug delivery system (CODES^{TM}). In this Pellets cores containing lactulose or mannitol were prepared by extrusion/spheronization and coated with an acid soluble polymer (Eudragit E100), Hydroxypropylmethyl cellulose (HPMC) and an enteric coat (Eudragit FS 30D) sequentially. *In vitro* drug release of coated pellets was studied using USP dissolution apparatus type II in buffers of pH 1.2 (2 hrs), pH of 7.4 (4 hrs) and pH of 6.8 containing 8% rat cecal contents (RCC) (18 hrs). The efficacy of the optimized formulation (containing 50% lactulose coated with Eudragit E (30% w/w) and Eudragit FS 30D (12% w/w)) was evaluated against 2, 4, 6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats.

Hangargekar Sachin et al [56] has formulated colon targeted tablets of Secnidazole with Xanthan gum as matrix carrier. Initially granules were prepared and evaluated for various rheological characteristic. *In vitro* release study was conducted for all formulations in USP XXIV 24 type I basket apparatus in different pH medias as follows, the tablets were put in the jars containing 900 ml of dissolution medium was stirred at 100 rpm at 37 ± 0.5^0 C for first 2 hour jar was filled with pH 1.2 dissolution fluid after 2 hour jar was filled with pH 7.4 for a period of 5 hour further jar assembly was lifted and dissolution fluid was replaced with pH 6.8 for a period of 24 hours, Samples were collected at the 1 hour time interval and studied for absorbance. Results were concluded that the formulations containing 25% w/w Xanthan gum showed maximum drug release in colonic environment.

Jinhe Li et al [57] has studied Dissolution Behavior for a Colon-Specific Drug Delivery System (CODES™) in Multi-pH Media using acetaminophen (APAP) as a model drug. Release profiles in artificial gastric fluid (pH 1.2), intestinal fluid (pH 6.8), and pH 5.0 buffer were determined. As expected, the percent release of APAP from coated core tablets was highly pH dependent. A release profile exhibiting a negligible release in pH 1.2 and 6.8 buffers followed by a rapid release in pH 5.0 buffer was established. It was interesting to note that there was a close similarity (f_2 = 80.6) between the release profiles at dip speed 5 dpm and paddle speed 100 rpm. In addition, the release rate was reduced significantly with the increase in acid-soluble Eudragit E coating levels, but lactulose loading showed only a negligible effect. In conclusion, the

established reciprocating cylinder method at lower agitation rates can give release profiles equivalent to those for the paddle procedure for CODES™ drug pH-gradient release testing

M. Zahirul I. Khan et al [58] has investigated lactose-based placebo tablets coated with various combinations of two methacrylic acid copolymers, EudragitL100-55 and EudragitS100, by spraying from aqueous systems. The Eudragit L100-55 EudragitS100 combinations (w/w) studied were 1:0, 4:1, 3:2, 1:1, 2:3, 1:4, 1:5 and 0:1. The coated tablets were tested *in vitro* for their suitability for pH dependent colon targeted oral drug delivery. The same coating formulations were then applied on tablets containing mesalazine as a model drug and evaluated for *in vitro* dissolution rates under various conditions. Dissolution studies performed on the mesalazine tablets further confirmed that the release profiles of the drug could be manipulated by changing the Eudragit L100-55 and Eudragit S100 ratios within the pH range of 5.5 to 7.0 in which the individual polymers are soluble respectively, and a coating formulation consisting of a combination of the two copolymers can overcome the issue of high gastrointestinal (GI) pH variability among individuals. The results also demonstrated that a combination of Eudragit L100-55 and Eudragit S100 can be successfully used from aqueous system to coat tablets for colon targeted delivery

Manish Neekhra et al [59] has investigated the fabrication of the novel colon drug delivery system of ketorolac tromethamine capsule. Hard gelatin Capsules were fabricated using combination of both microflora activated natural polysaccharide (guar gum) and pH-dependent polymer (cellulose acetate phthalate). *In vitro* drug release studies revealed that Ketorolac tromethamine granules bearing hard gelatin capsules coated with cellulose acetate phthalate released drug only at higher pH (6.8 PBS), *in vitro* drug release studies showed that capsules were retained in stomach pH (1.2 N HCl) with-out any drug release, whereas minimal amount of drug release in small intestine pH (7.4 PBS) and almost complete drug release showed in the colonic pH (6.8 PBS).

Lanjhiyana S.K et al [60] has investigated that the colon is an effective drug delivery site for local colorectal cancer treatment using 5-Fluorouracil (5-FU) as model drug based on oral pulsatile release technology. Formulations coated with enteric Eudragit-S100 and swellable hydroxyl propyl methyl cellulose (HPMC) to a varying coat thickness of 2:4, 4:2, 3:4 and 4:3, were tested for in-vitro drug dissolution with various simulated fluids of stomach (pH 1.2), small intestine (pH 7.2) and colon (pH 6.8 The optimized enteric coated formulations containing 30% guar gum released only 5-12% approximately of 5-FU during 5 h dissolution studies in hostile physiological environment of stomach (pH 1.2) and small intestine (pH 7.2).

Francesca Maestrelli et al [61] has studied the Enteric-coated calcium pectinate microspheres (MS) aimed for colon drug delivery has been developed, by using theophylline as a model drug. Enteric coating with Eudragit S100 enabled maintenance of MS integrity until its expected arrival to colon. Unexpectedly, addition of pectinolytic enzymes to the colonic medium did not give rise to selective enzymatic degradation of MS. Notwithstanding this unforeseen result, coated MS prepared at 2.5% w/v CaCl2 concentration were able to adequately modulate drug release through a mixed approach of pH and transit time control, avoiding drug release in the gastric ambient, and reaching the colonic targeting where 100% release was achieved within less than 24 h.

Soravoot Rujivipat and Roland Bodmeier [62] has developed pH-erosion-controlled compression-coated tablets for potential colonic drug delivery based on compression-coatings of powder blends of the enteric polymer Eudragit L100-55 and the extended release polymer Ethylcellulose. Tablet cores containing model drugs of varying solubility's (Acetaminophen, Carbamazepine and Chlorpheniramine maleate) were compression coated with different ratios of Eudragit L100-55: Ethylcellulose 10cP FP. All drugs were released in a pulsatile fashion in higher pH-media after a lag time, which was controlled by the erosion properties of the Eudragit L: Ethylcellulose compression-coating. In conclusion, tablets compression-coated with blends of Eudragit L and Ethylcellulose resulted in excellent release properties for potential targeting to the lower intestinal tract with no release in lower pH-media and rapid release after a controllable lag time in higher pH-media.

Takashi Ishibashi et al [63] have investigated a new capsule-type dosage form for colon-targeted delivery of drugs. The system was designed by imparting a timed-release function and a pH-sensing function to a hard gelatin capsule coated with a three-layered film consisting of an acid-soluble polymer, a water-soluble polymer, and an enteric polymer. In order to find the suitable formulation, various formulation factors were investigated through a series of *in vitro* dissolution studies. As a result, it was found that: (1) various organic acids can be used for this system; (2) a predictable timed-release mechanism of a drug can be attained by adjusting the thickness of the Eudragit E layer; and (3) the outer enteric coating with HPMC®-AS provided acceptable acid-resistibility. All these results suggested that this approach can provide a useful and practical means for colon-targeted delivery of drugs.

Xiaxia Shen et al [64] developed Eudragit L 100-55 nanofibers loaded with diclofenac sodium (DS) were successfully prepared using an electrospinning process, and characterized for structural and pharmacodynamic properties. The influence of solvent and drug content on fiber formation and quality was also investigated. Fiber formation was successful using a solvent mixture 5:1 (v/v) ethanol: DMAc. XRD and DSC analysis of fibers confirm electron microscopic evidence that DS is evenly distributed in the nanofibers in an amorphous state. FTIR analysis indicates hydrogen bonding occurs between the drug and the polymer, which accounts for the molecular integration of the two components. *In vitro* dissolution tests verified that all the drug-loaded Eudragit L 100-55 nanofibers had pH-dependent drug release profiles, with limited, less than 3%, release at pH 1.0, but a sustained and complete release at pH 6.8. This profile of properties indicates drug-loaded Eudragit L 100-55 nanofibers have the potential to be developed as oral colon-targeted drug delivery systems.

R.Thiruganesh et al [65] has developed a single unit, site-specific matrix tablets of Aceclofenac allowing targeted drug release in the colon with a microbially degradable polymeric carrier, Chondroitin Suphate (CS) and to coat the optimized batches with a pH dependent polymeric coating solution containing Eudragit L 100 and S 100 (1: 4). The tablets were prepared by wet granulation using starch mucilage as a binding agent and HPMC K-100 as a swellable polymer. The tablets were tested for their *in-vitro* dissolution characteristics in various simulated gastric fluids for their suitability as a colon-specific drug delivery system. The dissolution data demonstrates that the 10% w/w increase in coating level of the pH dependent polymer (Eudragit

L-100 and Eudragit S-100 in a ratio of 1: 4 prevented the drug release in the simulated gastric fluid (pH 1.2-SGF) and the simulated intestinal fluid (pH 7.4-SIF).

Purushothaman M and Vijay Ratna.J [66] has investigated the matrix formulations taken for compression and spray coating to test the suitability for targeted drug delivery to the colon. The release kinetics of the formulations was calculated. All the Matrix, Compression coated and Spray coated formulations were showed the desired physicochemical properties as per the official limits. Based on the drug release study in pH 1.2 (0.1N HCl), Phosphate buffer pH 7.4 and 6.8, the three TGT F8, TGTE F20, and TGTES F32 were found to be best and they were taken for further release study in phosphate buffer pH 6.8 with rat cecal content (4% w/v). The formulations TGTE F20 ((r2 - 0.9986, n - 1.0601) and TGTES F32 (r2 -0.9974, n - 1.1501) were shown zero order release in terms of its kinetic release.

Mayur M. Patel et al [67] investigated the colon specific drug delivery system (CDDS) which is designed such that the innermost part consists of a core tablet of mesalamine which is then compression coated with a pH-independent hydrophilic polymer (Hydropropylmethyl cellulose). This is then coated with a pH-dependent methacrylic acid copolymer (Eudragit® S100). The concentration (coating level) of Eudragit® S100 was optimized to provide an enteric coat that allows the tablet to pass intact through the stomach and is targeted to the colon. The coating thickness and grades of HPMC were optimized to set a desired lag time in the intestine. From the *in vitro* evaluation it can revealed that the developed CDDS can exhibit site-specific drug targeting to the colon.

Mohanad naji sahib[68] formulated prednisolone as an oral modified release tablet for colonic targeting. The formula containing 1% Eudragit RS PM was the best with regard to 100% release of drug in comparison with other concentrations and other retardant types. Avicel was used as a canalizing agent, and the results showed that the formula containing 30% Avicel PH 302 demonstrated faster release. Eudragit S 100 provided the best release of drug in phosphate buffer, pH 7.4. The effect of the percent of binding agent polyvinylpyrrolidone (PVP) (5%, 10%, and 15%) was studied, and the best results were obtained with a concentration of 10%. The trials in this study successfully formulated prednisolone-modified release tablets (coated matrix) using a wet granulation method as a potential colon delivery syst

Obitte NC et al [69] has evaluated the *in vitro* effect of, the percentage of surface area of capsule surface coated with Landolphia owariensis latex (LOL), particle size of granules, and %w/w of matrix former (methylcellulose) on the release of Metronidazole from coated hard gelatin capsules for possible delivery to the colon. Metronidazole granules were prepared by the wet granulation technique and appropriately encapsulated prior to primary coating of capsule with Eudragit® L-100 and secondary coating with LOL. Capsules having primary coating of Eudragit® L- 100 were coated with LOL atop 50% or 85% capsule surface. *In vitro* drug release was carried out sequentially in media of pH 1.2(0.1N HCl), 6.8 and 7.4(phosphate buffer solution) respectively. Results showed that the greatest quantity of drug release took place at pH 7.4 at 20 h

Sheth Zankhana et al [70] presented that improvement in the efficacy, reduction in toxicity and enhancement of therapeutic index of 5-fluorouracil.Biodegradable microparticulate delivery system of 5-fluorouracil has been developed by solvent evaporation technique by using polymethacrylate polymers like eudragit L100, eudragit S100, eudragit P4135F and methylcellulose. Four different formulations were prepared by using these polymers in drug to polymer ratio of 1:2. The formulations were evaluated with respect to particle size analysis, entrapment efficiency, *in vitro* drug release studies, *in vivo* drug targeting studies and stability studies. The formulated magnetic microspheres were found to be spherical with average particle size of 3-12 µm in diameter and incorporation efficiency up to 78.80%. *In vitro* drug release after 12 hr was 86.41 %, 92.84 %, 79.88 % and 82.38 % for formulation F1, F2, F3 and F4 respectively. Formulation F2 with highest drug content was selected for *in-vivo* drug targeting studies. The average targeting efficiency of drug loaded microspheres was found to be 26.16 % of the injected dose in liver, 11.40 % in lungs, and 15.08 % in spleen, whereas the concentration of pure drug was 15.52 % in liver, 9.0 % in lungs, and 9.50 % in spleen. These results reveal that the drug loaded microspheres showed preferential drug targeting to liver followed by spleen and lungs. Stability studies revealed that 4º C is the most suitable temperature for storage of 5 - fluorouracil loaded microspheres. Overall, this study showed that the 5 - fluorouracil can be formulated in a microparticulate drug delivery system by using various polymers and it showed significant prolonged drug release.

Jatin A Popat [71] presented that the core tablets of Salbutamol Sulphate were prepared using wet granulation containing a superdisintegrant. Eudragit S100 and Eudragit L100 were used as pH dependent polymers for coating the core tablet which were filled in to the capsule. The ratio of Eudragit S100 and Eudragit L100 and the coating level was optimized using 32 full factorial designs. Factors studied in design were percentage of Eudragit S100 in combination with Eudragit L100 and the effect of coating level on In-vitro drug release. Dissolution studies at different pH (1.2, 5.5, 6.8 and 7.4) showed that drug release in colon could be modulated by optimizing the concentration of Eudragit L100: Eudragit S100 (1:2). The study showed that, lag time prior to drug release was highly affected by the coating level. The dissolution data revealed that the level of coating and the ratio of polymers are very important to achieve a optimum formulation.

Gauri Bhawna et al [72] has developed the Matrix system of Tinidazole by using swellable pH dependent polymer like Hydroxypropylmethylcellulose (HPMC-K4M) and guar gum and methacrylic acid polymers like Eudragit FS 30D. Prepared tablets were compression coated in order to overcome variability in gastric emptying time and delay in release, to reduce the gastric side effects and to provide prolonged localized action in colon. The coated formulations were evaluated for dissolution rates under stomach and simulated intestinal conditions in presence of rat cecal content medium. *In-vivo* gamma scintigraphy study was also performed on (F3) formulation in healthy human volunteers using Tc-99m as a tracer medium. *In-vitro* drug release studies and *In-vivo* gamma-scintigraphic studies using Tc-99m as a tracer indicated that greater portion of Tinidazole was released in the large intestine and drug level was maintained in blood for 20h.

Upendra Nagaich et al [73] has developed the colon specific sustained release matrix tablets of an antifilarial drug diethylcarbamazine citrate (DEC). The colon targeted matrix tablet was prepared by wet granulation technique using different percentage of guar gum as matrix carrier and coated with Eudragit L-100. The dissolution study of DEC matrix tablet was performed in simulated colonic fluids (phosphate buffer pH 6.8) was found to be 94% and in simulated colonic fluids (rat caecal content medium) was 98% after degradation into 2-3 pieces at the end of the 24 h study. The result of the studies showed that colon targeted matrix tablet containing 45% of guar gum was most likely to provide targeting of DEC for local action in the colon. The colon

targeted matrix tablet of DEC showed no change either in physical appearance, drug content or in dissolution pattern after storage at 300± 20C / 65 ± 5% RH for 2 month. FT-IR spectrum showed no interaction between DEC and guar gum.

Rupesh S. Kamble [74] has preseted that Ketorolac Tromethamine direct compression method was used for the preparation of fabricated batches and EudragitL100 is used as coating polymer for enteric coating. *In vitro* release profiles of batches F1-F4 shows that Ketorolac Tromethamine in drug polymer ratio with Guar gum, Xanthan Gum, Ethyl cellulose and Sodium alginate give 79.32%, 91.52%, 88.35% and 92.19% drug release respectively in 12 hours. *In vitro* release profile of batches F5-F8 shows that Ketorolac Tromethamine in ratio 1:4 with Guar gum, Xanthan Gum, Ethyl cellulose and Sodium alginate gives release of 85.21%, 95.52%, 93.50%, 97.24% respectively in 12 hours. *In vitro* release profile of batches F9-F12 shows that Ketorolac Tromethamine in ratio 1:3 with Guar gum, Xanthan Gum, Ethyl cellulose and Sodium alginate gives release of 89.50%, 98.25%, 95.22%, 100.27% respectively in 12 hours. All the batches showed no drug release in first two hours in hydrochloric buffer of pH 1.2 and then showed higher increase in phosphate buffer of pH 6.0 up to 12 hours. All these batches follow near zero order kinetic.

M.S. Shaikh [75] has developed the oral colon targeted drug delivery system for Nimesulide. CODES^{TM} tablets were prepared by tabletting Nimesulide and lactulose, followed with film coating of Eudragit. The prepared tablets were evaluated on the basis of *in vitro* dissolution study and *in vivo* disintegration study was performed by gamma scintigraphic evaluation in rats. The onset of Nimesulide release was found to dependent on the coating level of Eudragit E, and at Eudragit E coating level of 8% (coating weight gain), the onset of *in vitro* drug release was found to be optimum. When the same was subjected on scintigraphic evaluation for *in vivo* disintegration study, there was a reasonable agreement between the *in vitro/in vivo* data. It is concluded that Nimesulide can be targeted to hindgut by a novel approach of CODES^{TM}.

K.Chandramohan et al [76] has investigated, GRAS (Generally regarded as safe) Polysaccharides such as Guar gum, Pectin, Dextrin were used as carriers to formulate a colon specific drug delivery system for Tinidazole at different ratios of drug: polymer (1:1.5, 1:1.0, and 1:0.5) by wet granulation method. The formulated tablets were evaluated. *In vitro* release studies were

performed for their suitability as colon specific drug delivery system. Of the thirteen formulations (F1-F13) best formulations were selected which shows restricted drug release in small intestine(13.7-36.2%) and which shows more release (56.6-99.9%) in colonic environment The selected formulations were enteric coated with Eudragit L-100, to target them to colon. The enteric coated tablet (ETF1-ETF6) shows 89.8-96.2% of drug at the end of 10^{th} hour in presence of rat caecal medium.

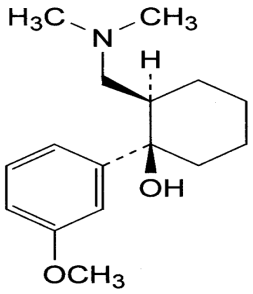
S. C. Dhawale et al [77] has given the treatment of colon cancer 5-Fluorouracil is a candidate to be delivered orally to the colon; pH - sensitive polymers Eudragit S 100 and L 100 were used to prepare microspheres by a simple oil /water emulsification process. Process parameters were analyzed in order to optimize the drug loading and release profiles. In further attempts mixtures with Eudragit S100 and L100 were prepared to prolong drug release. Scanning electron microscopy permitted a structural analysis. Eudragit S100, pure or in mixture, was found to retain drug release at pH 4.5 lower than 41% within 6 h. At pH 7.4, nearly immediate release (within 30 min) was observed for pure S100, while mixtures enabled to prolong the release slightly. Analysis of the morphology led to an inhomogeneous polymer distribution of S100 and L 100 throughout the particle core. However, the formulation proved its applicability in-vitro as a promising device for pH-dependent colon delivery of 5-fluorouracil.

Drug Polymer Profile

3.DRUG PROFILE

TRAMADOL HCL[31, 32, 33]

1. STRUCTURE :



and enantiomer , HCl

2. CHEMICAL NAME : (1RS, 2RS)-2-[(Dimethylamino) methyl]-1(3-Methoxyphenyl) Cyclohexanol Hydrochloride

3. MOLECULAR WEIGHT : 299.8

4. MOLECULAR FORMULA : $C_{16}H_{26}ClNO_2$

5. APPEARENCE : White or almost white, crystalline powder

6. MELTING POINT : 178°C (352.4°F) - 181 C

7. FUNCTIONAL CATRGORY: Opioid receptor agonist; Noradrenalin reuptake inhibitor; analgesic

8. SOLUBLITY : Freely soluble in water and in methanol, Very slightly soluble in acetone

9. STORGAGE : Protected from light.

10. HALF LIFE : Approximately 5–6 hours

Indications and Usage for Tramadol HCl Tablets [33]

Tramadol hydrochloride tablets are indicated for the management of moderate to moderately severe pain in adults.

Contraindications [47]

Tramadol hydrochloride should not be administered to patients who have previously demonstrated hypersensitivity to tramadol, any other component of this product or opioids. Tramadol hydrochloride is contraindicated in any situation where opioids are contraindicated, including acute intoxication with any of the following: alcohol, hypnotics, and narcotics, centrally acting analgesics, opioids or psychotropic drugs. Tramadol hydrochloride may worsen central nervous system and respiratory depression in these patients.

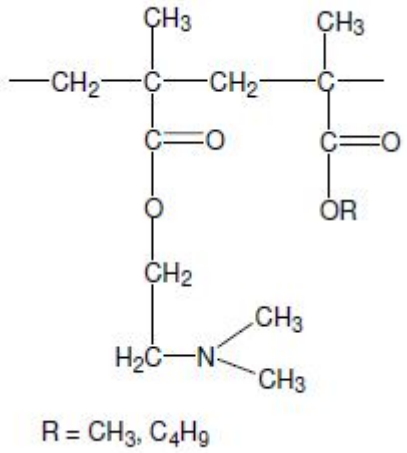
Table 7: Available dosage forms in market [33]:

Tramadol Hydrochloride

Routes	Dosage Forms	Strengths	Brand Names	Manufacturer
Oral	Tablets, extended-release	100 mg*	Tramadol Hydrochloride Extended-Release Tablets	
			Ultram ER	PriCara
		200 mg*	Tramadol Hydrochloride Extended-Release Tablets	
			Ultram ER	PriCara
		300 mg	Ultram ER	PriCara
	Tablets, film-coated	50 mg*	Tramadol Hydrochloride Tablets	
			Ultram (scored)	PriCara

EUDRAGIT E 100 [32,33,34]

1.STRUCTURE :



R = CH_3, C_4H_9

2. MOLECULAR WEIGHT : 1, 50, 000.

3. APPEARENCE : colorless to yellow tinged granules with a Characteristic amine-like odour

4. FUNCTIONAL CATRGORY : EUDRAGIT E 100 is a cationic copolymer Based on dimethylaminoethyl Methacrylate and neutralmethacrylic esters

5. SOLUBLITY : 1 g of EUDRAGIT E 100 dissolves in 7 g Methanol, ethanol, isopropyl alcohol, Acetone, Ethyl acetate methylene chloride or 1 N hydrochloric acid to give clear to slightly Cloudy solutions

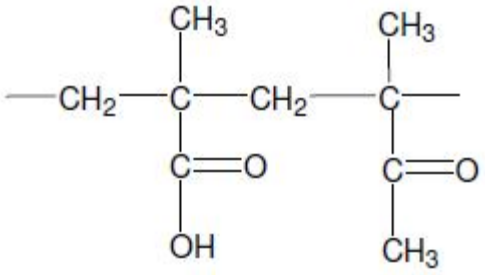
6. FILM FORMATION : When the Test solution is poured onto a Glass plate, a clear film forms upon Evaporation of the solvents

7. STORGAGE : Protected from light.

8. LOSS ON DRYING : EUDRAGIT E 100 max. 2.0 % according to "Dry substance / Residue on evaporation"

EUDRAGIT S 100 [32,33,34]

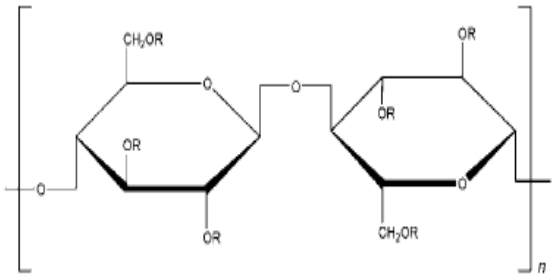
1.STRUCTURE



2. MOLECULAR WEIGHT	:	1, 35,000.
3. APPEARENCE	:	White powders with a faint characteristic Odour
4. FUNCTIONAL CATRGORY	:	EUDRAGIT S 100 is anionic Copolymers based on methacrylic acid and Methyl methacrylate
5. SOLUBLITY	:	1g EUDRAGIT S 100 dissolves in 7g Methanol, ethanol, in aqueous Isopropyl Alcohol and acetone containing approx 3% Water as well as in 1 N sodium hydroxide to give clear to slightly cloudy solutions. Eudragit S100 is practically insoluble in ethyl acetate, methylene Chloride, petroleum ether and water
6. FILM FORMATION	:	When the Test solution is poured onto a Glass plate, a clear film forms upon Evaporation of the solvents
7. STORGAGE	:	Protected from light.
8. LOSS ON DRYING	:	Max. 5.0 % according to "Dry substance / Residue on evaporation"

HYDROXY PROPYL METHYL CELLULOSE [32, 33, 34]

1.STRUCTURE :



Where R is H, CH3, or CH3CH (OH) CH2

2. CHEMICAL NAME : Cellulose hydroxypropyl methyl ether

3. MOLECULAR WEIGHT : 10 000–1 500 000.

4. MOLECULAR FORMULA : $C_{16}H_{26}ClNO_2$

5. APPEARENCE : odorless and tasteless, white or creamy white
Fibrous or granular powder

6. MELTING POINT : browns at 190–2008C; chars at 225–2308C.
Glass transition temperature is 170–1808C.

7. FUNCTIONAL CATRGORY : Coating agent; film-former; rate-controlling
Polymer for sustained release; stabilizing

Agent; suspending agent; tablet binder;
Viscosity-increasing agent

8. SOLUBLITY : soluble in cold water, forming a viscous Colloidal Solution; practically insoluble in Chloroform, ethanol (95%), and ether, but Soluble in mixtures of ethanol and Dichloromethane, mixtures of methanol and Dichloromethane and mixtures of water and Alcohol Certain grades of hypromellose are Soluble in aqueous Acetone solutions, mixtures of dichloromethane and Propan-2 ol and other organic solvents

9. STORGAGE : Well-closed container, in a cool, dry place.

Materials and Methods

Table 8: List of excipients used and their sources

MATERIALS	SOURCES
Tramadol hydrochloride	NuLife pharmaceuticals pune
Spray dried lactose	Twilight Litaka pharmaceuticals pune
Crosspovidone	Twilight Litaka pharmaceuticals pune
Avicel pH 102	NuLife pharmaceuticals pune
Aerosol	Adinath Chemicals Mumbai
Talc	Caltron Clays & Chemicals Private Limited Mumbai
Magnesium stearate	Sankalp Organics Private Limited Mumbai
Eudragit E 100	Twilight Litaka pharmaceuticals pune
HPMC 2910	Cipla Ltd, Goa (Adinath Chemicals Mumbai)
Eudragit S 100	Cipla Ltd, Goa
Isopropyl alcohol	NuLife pharmaceuticals pune
Polyethylene glycol (PEG) 400	NuLife pharmaceuticals pune
Propylene glycol	Shivam Industries, Mumbai Mumbai

Table 9: List of equipments used and sources:

INSTRUMENTS	SOURCES
Single pan analytical balance	ANAND Mumbai
Tablet punching machine	CADMACH 16 station single rotary tablet punching machine
Tablet hardness tester	Pfizer tablet hardness tester
Friabilator	VEEGO friabilator
Disintegration apparatus	VEEGO DIGITAL tablet Disintegration apparatus
Magnetic stirrer	KMS- 400 Remi
Coating machine	Bohle coating machine
Dissolution apparatus	ELECTROLAB tablet dissolution tester USPXXIV TDT.O8 L

5. METHODOLOGY

5.1 Determination of λ max:

10mg/ml solution of Tramadol hydrochloride was taken in specific buffer solution (pH1.2, pH7.4, pH6.8) using serial dilution technique and scanned in range 200-400nm using UV spectrometer to find out the wavelength of maximum absorbance.

5.2 Construction of standard curve:

100 mg of Tramadol hydrochloride was taken in 100 ml of specific buffer solution (pH1.2, pH 7.4, and pH 6.8) dissolved properly by shaking and kept on sonicater for 10 min 1 ml of the solution was withdrawn and diluted with 100 ml of respective buffer solution and from the stock solution 2, 4, 6, 8 and 10ml of solution was transferred into 10 ml volumetric flask and the volume was made up to 10 ml to produce 2, 4, 6, 8 and 10 µg/ml solution respectively. Absorbance was measured in UV spectrometer at 271nm wavelength.

5.3 Identification test [51]:

Weigh about 0.2 gm of test sample add 2ml of water. Acidify with dilute Nitric acid. Add 0.5 ml of silver nitrate solution shake and allow to stand; A curdy white precipitate is formed which is insoluble in Nitric acid but soluble after being well washed with water in dilute ammonia solution from which it is reprecipitated by the addition of dilute Nitric acid.

5.4 Preformulation studies [32, 35, 36]:

Prior to development of a new dosage form with a drug moiety, it is essential that certain fundamental physical and chemical properties of the drug candidate and excipients are determined. The preformulation parameters like bulk density, tapped density, compressibility index Hausner ratios etc. were determined as per USP procedure. The procedures followed for various tests are given below:

5.4.1 Bulk Density [36]

The powder blend under test was screened through sieve no. 18 and the sample equivalent to 10 g was accurately weighed and filled in a 25 ml graduated cylinder and the powder was leveled and the unsettled volume, V_0 was noted.

The bulk density was calculated in gm / cm^3 by the formula,

Bulk Density (δ_0) = M / V_0 (1)

M = Mass of powder taken

V_0 = Apparent unstirred volume

5.4.2 Tapped Density [36]

The powder blend under test was screened through sieve no.18 and the weight of sample equivalent to 10 g was filled in 25 ml graduated cylinder. The mechanical tapping of the cylinder was carried out using tapped density tester at a nominal rate of 300 drops per minute for 500 times initially and the tapped volume V_0 was noted. Tapping was proceeding further for an additional tapping 750 times and tapped volume, V_b was noted. The tapping was continued until the difference between two successive tapped volumes was less than 2%. The tapped density was calculated in g / cm^3 by the formula,

Tapped density (δ_t) = M / V_f (2)

M =Weight of sample powder

V_f = Tapped volume

5.4.3 Carr's Index [35]

The bulk density, cohesiveness of the material, surface area, size and shape and the moisture content influences the Compressibility index. The compressibility index is determined from the bulk volume and tap volume. The basic method used for the determination of compressibility index is to measure the bulk volume and the final tapped volume after a fixed number of tapping until no change in volume occurs. It is represented in percentage.

The Carr's index of the powder was determined by using formula:

Carr's index (%) = [(TBD - LBD) × 100]/TBD **(3)**

Where,

LBD = weight of the powder/volume of the packing

TBD = weight of the powder/tapped volume of the packing

Table 10: Standard values for Carr's index:

Carr's index %	Type of flow
5-15	Excellent
12-18	Good
18-23	Satisfactory
23-35	Poor
35-38	Very poor
>40	Extremely poor

5.4.4 Hausner ratio [44]:

Hausner's ratio is the ratio of the initial volume of the powder mass to the final volume of the powder mass obtained after the specified number of tapping.

Hausner ratio is calculated from the formula,

Hausner ratio= ($\delta t / \delta_0$) **(4)**

δ_t = Tapped density

δ_0 = Bulk density

Table 11: Scale of Flowability based on Hausner's Ratio

HAUSNER'S RATIO	FLOW CHARACTER
1-1.11	Excellent
1.12-1.18	Good
1.19-1.25	Fair
1.26-1.34	Passable
1.35-1.45	Poor
1.46-1.59	Very poor
>1.60	Very, very poor

5.4.5 Angle of Repose [36]

Angle of repose is defined as maximum angle formed between surface of pile of powder and horizontal plane. It is determined by the funnel method. 10g of the powder was accurately weighed and taken in a funnel closed at the bottom with a cotton plug. Height of the funnel was adjusted such that the tip of the funnel just touches the apex of the heap of powder. The powder was allowed to flow through funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation no 5.

$\tan \theta = h/r$ **(5)**

Therefore, $\theta = \tan^{-1} h/r$

Where, θ = angle of repose, h = height of the cone and r = radius of the cone base

Table 12: Standard values for Angle of repose:

Angle of repose in degrees	Type of flow
<25	Excellent
25-30	Good
30-40	Satisfactory
>40	Poor

5.4.6 Compatibility Studies:

IR spectra matching approach was used for detection of any possible chemical interaction between drug and polymer. A pure drug, uncoated tablet and coated tablets were separately mixed with three suitable quantity of potassium bromide. About 100mg of mixture was compressed to form a transparent pellet using a hydraulic press at 6tons pressure .It was scanned from 4000 to 400 cm-1 in FTIR spectrometer. The IR spectrum of the physical mixture was compared with those of pure drug and polymers and matching was done to detect any appearance or disappearance of peaks. The IR spectrums of the tablets in the range of 4000 cm-1 to 400 cm-1 were taken by preparing dispersion in dry potassium bromide under the same operational conditions mentioned above.

5.5 Formulation of tablets for colon targeting:

The tablets for colon targeting was developed by preparing a core tablet containing 100mg of Tramadol HCl, which was the coated with pH sensitive polymers such as Eudragit S100, E100 and HPMC in three stages.

5.5.1 Preparation of core tablets of Tramadol HCl:

Core tablets of Tramadol HCl were prepared by direct compression method, as per the formula given in Table no 13.

Table 13: Formula used for core tablet:

INGREDIENTS	QUANTITY TAKEN (mg)
Tramadol hydrochloride	100
Spray dried lactose	208
Crosspovidone	22
Microcrystalline cellulose	150
Aerosil	20
Talc	25
Magnesium stearate	25

The ingredients required were weighed accurately and mixed thoroughly using a powder blender and sieved through sieve no.80. The powder blend was then lubricated with the addition of talc and magnesium stearate. The lubricated blend was then compressed into tablets by using a 16 station rotary tablet punching machine (Cadmach, Ahmedabad). The compression parameters used are given in Table no 14.

Table 14: Compression parameters:

NAME OF MACHINE	CADMACH 16 station tablet punching machine
NUMBER OF STATION	16
ROATION PER MINUTE	4-5
COMPRESSION PRESSURE	40-65 kilo Newton
PUNCH SIZE	5×18mm
TOOLING	B Tooling

5.6 EVALUATION OF CORE TABLETS:

5.6.1 Thickness testing [49]

The thickness of the matrix tablets was determined by using vernier calipers, and the results are expressed as mean values of 10 determinations.

5.6.2 Tablet Hardness [49]

The hardness of the tablet was measured by pfizer hardness tester. The tablets were held vertically in between the jaws, which were pressed with hand until the tablet broken. The reading was noted from needle which was expressed in kg. A total of three tables were tested and the mean values were reported.

5.6.3 Weight Variation [36]

Weight variation test for the core tablets was performed as per the IP procedure. Twenty tablets were weighed individually and the average weight was determined. The individual weight of all the twenty tablets was noted. The percentage deviation of the individual weights from the average weight was then calculated. Deviation should not exceed the values given in table no 15

Table 15: Percentage deviation allowed in weight variation:

AVERAGE WEIGHT OF TABLET	PERCENTAGE DEVIATION (±)
80 mg or less	10
More than 80 mg but less than 250mg	7.5
250 mg or more	5

5.6.4 Friability [36]

Friability was performed to evaluate the ability of tablet to withstand abrasion. VEEGO friabilator was used for testing the friability. Twenty tablets were weighed accurately and placed in the tumbling apparatus that revolves at 25 rpm. After 4 min., the tablets were weighed and the percentage loss in tablet weight was determined.

$$\% \text{ loss} = \frac{\text{Initial wt. of tablets - Final wt. of tablets}}{\text{Initial wt. of tablets}} \times 100 \quad (6)$$

5.6.5 Assay:

Standard preparation:

Weigh accurately 0.11 gm of Tramadol hydrochloride (working formula) in 10ml of volumetric flask which is previously dried with Methanol. Dilute to the mark with methanol to get final concentration i.e. 1.1mg/ml

Test preparation:

Weigh 20 tablets and powder them. Take powdered tablet equivalent to 0.011gm i.e. about 0.16gm in 100ml of beaker which is previously dried. Add 50ml of chloroform in it. Keep it on ultrasonic bath for 15-20 min with slight heating then filter the chloroform in another dry beaker and evaporate chloroform to dryness. After evaporation make dilution with methanol to 10ml by rinsing the beaker. The absorbance of the diluted solution was then measured at 271nm using methanol as the blank solution. The assay value is calculated by using the formula:

$$\textbf{Assay} = \frac{\textbf{Test reading}}{\textbf{Standard reading}} \times \frac{\textbf{Wieght of standard}}{\textbf{Weight of test}} \times \mathbf{100} \qquad \textbf{(7)}$$

5.7 Preparation of coated tablets:

The core tablets prepared were then coated with two different pH sensitive polymers, Eudragit E100 and S100 and by using HPMC as the barrier coating material. The sequence of coating is as follows;

1) **10% Eudragit E100 [50] coated immediately over the core tablet**
2) **10% HPMC was then coated over the E100 coating as a barrier coat.**
3) **6% Eudragit S 100 [35] was coated as the outer most coating.**

- **EUDRAGIT E100**:- Cationic polymer carries amino groups insoluble in neutral medium of saliva but dissolves in acid medium of gastric fluid. The polymer becomes permeable at a pH of more than 5.5
- **EUDRAGIT S100**:-Anionic polymer carry carboxyl groups insoluble in acid medium i.e. resistant to gastric fluid and dissolves in alkaline medium of intestine
- **HPMC**:-This layer acts as barrier layer and prevents the possible interaction between EudragitE100 & Eudragit S100.

Table 16: Coating composition for the three polymers:

POLYMERS	**SOLVENT USED**	**PLASTICIZERS USED IN %**
EUDRAGIT E-100	ISOPROPYL ALCOHOL	---------
HPMC	WATER	PROPYLENE GLYCOL15% + TALC 10%
EUDRAGIT S-100	ISOPROPYL ALCOHOL	PEG-400 2%

Table 17: Coating polymer ratio:

POLYMER	%WEIGHT GAIN (COATING LEVEL)								
	F-1	**F-2**	**F-3**	**F-4**	**F-5**	**F-6**	**F-7**	**F-8**	**F-9**
EUDRAGIT S-100	3	3	3	6	6	6	9	9	9
HPMC	5	5	5	5	5	5	5	5	5
EUDRAGIT E-100	3	6	9	3	6	9	3	6	9

5.8 Coating method:

Coating was done by three successive layers in coating pan by spray pan coating method,

5.8.1 Coating of Eudragit E100 solution:

100 ml of Isopropyl alcohol was taken in clean and dried beaker and kept on magnetic stirrer. Accurately weighed amount (10gm) of EUDRAGIT E 100 was slowly added in beaker and beaker was closed with a polyethylene cover to avoid evaporation during stirring. The coating solution was kept for 30 min and this (10% Eudragit E100 solution) was sprayed on tablet by using 12" coating pan with following parameters given below in table no 18.

5.8.2 Coating of HPMC barrier layer:

Second barrier layer coat was given to avoid any possible interaction between Eudragit E100 and Eudragit S100 since they are oppositely charged polymers and they may have attraction towards each other. The coating solution was prepared by taking 10 gm of HPMC2910 and added slowly in 100ml (10% of HPMC2910 solution) of water and volume was adjusted after adding plasticizers, propylene glycol 15% and Talc 10%. Then this coating polymer was stirred for 5-7 min, filtered and then sprayed on the same batch of tablet previously coated with Eudragit E100.

5.8.3 Coating of Eudragit S100:

Third coating layer was prepared by taking 6gm of Eudragit S100 in a beaker (which is dried) containing 100 ml of Isopropyl alcohol with 2% of propylene glycol as a plasticizer and stirred in magnetic stirrer for 30 min and beaker was closed with a polyethylene cover to avoid evaporation during stirring. This (10% Eudragit S100 solution) was sprayed on tablet by using 12" coating pan with following parameters given below in table no18.

Table 18: Coating parameters:

AIR PRESSURE (kg/cm^2)	1.5-2.5	
PAN SPEED (RPM)	4-5	
BED TEMPERATURE	$35\text{-}40^0$ C	
NUMBER OF BAFFELS	2	
NUMBER OF SPRAYING GUN	1	
GUN DISTANCE (cm)	FOR HPMC COAT (aqueous)	FOR EUDRAGIT COAT (non aqueous)
	20-25	15-18

5.9 Evaluation of coated tablets:

The coated tablets were subjected to various evaluation tests similar to that of the uncoated tablets, such as thickness, hardness, friability, weight variation, assay etc. Apart from the above studies, the tablets were also subjected to *in vitro* drug dissolution studies using different mediums simulating the GIT environment.

5.9.1 *In vitro* drug release studies [37]

In vitro drug release studies were carried out using USP XXIV dissolution test apparatus Type II, paddle apparatus (50 rpm, 37+ 0.5^0 C). In vitro release study for enteric coated tablets was carried out by keeping the tablets for 2 h in pH1.2 (900 ml) acid buffer solution, simulated gastric fluid (SGF). The dissolution medium was then replaced with pH 7.4 phosphate buffer solution (900 ml), simulated intestinal fluid (SIF), and tested for 3 h, which was later replaced by pH 6.8 phosphate buffer solution (900 ml), simulated colonic fluid (SCF), and tested for release for 19 h. The drug release at different time intervals was analyzed by UV double beam spectrophotometer (Electrolab TDT-08 L) at 271 nm

5.9.2 Release kinetic analysis

To study the release kinetics, data obtained from *in- vitro* drug release studies were plotted in various kinetic models: zero order (Equation 8) as cumulative amount of drug released vs. time, first order [78] (Equation 9) as log cumulative percentage of drug remaining vs. time, and Higuchi's model [79] (Equation 10) as cumulative percentage of drug released vs. square root of time.

$$C = K_0 t \qquad (8)$$

Where

K_0 : is the zero-order rate constant expressed in units of concentration/time

t: is the time in hours.

A graph of concentration vs. time would yield a straight line with a slope equal to K_0 and intercept the origin of the axis.

$$\mathbf{LogC = LogCo - kt/2:303} \qquad \mathbf{(9)}$$

Where

C_0: is the initial concentration of drug,

K: is the first order constant, and t is the time.

$$\mathbf{Q = Kt^{1/2}} \qquad \mathbf{(10)}$$

Where

K: is the constant reflecting the design variables of the system

t: is the time in hours.

Hence, drug release rate is proportional to the reciprocal of the square root of time.

To evaluate the drug release with changes in the surface area and the diameter of the particles/tablets, the data were also plotted using the Hixson-Crowell cube root law [80]:

$$\mathbf{3\sqrt{Q_0} - 3\sqrt{Q_t} = kHC - t} \qquad \mathbf{(11)}$$

Where Q_t is the amount of drug released in time t,

Q_0 is the initial amount of the drug in the tablet,

And KHC is the rate constant for the Hixson-Crowell rate equation, as the cube root of the percentage of drug remaining in the matrix vs. time.

Mechanism of Drug Release [81, 82]

Higuchi's plot has indicated that diffusion might be one of the prominent mechanisms influencing the drug release. To evaluate the mechanism of drug release from Tramadol tablets, data of drug release were plotted in Korsmeyer et al's Equation 12 as log cumulative percentage of drug released vs. log time, and the exponent n was calculated through the slope of the straight line.

$$\mathbf{Mt - M\infty = Kt^n} \qquad \mathbf{(12)}$$

Where

Mt/M∞ is the fractional solute release,

t is the release time,

K is a kinetic constant characteristic of the drug/ polymer system, and n is an exponent that characterizes the mechanism of release of tracers.

5.9.3 Stability studies:

Stability study was done to check out the quality of drug substance or product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light; to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage condition.

Here the tablets were loaded at accelerated condition at 40^0C±2 ^{0} and 75% RH in a stability chamber. Sample were withdrawn after 30 and 60 days and analyzed suitably for the drug content and dissolution characteristics.

Results and Discussion

6. RESULTS AND DISCUSSION

6.1 Determination of λ max:

The wavelength showing maximum absorbance (λ max) for Tramadol hydrochloride was determined by scanning the standard stock solution of the drug using UV visible spectrophotometer. The λ max was found to be 271nm for Tramadol HCl which is in accordance with the data available in literature.[83]

6.2 Calibration curve:

A standard calibration curve of Tramadol HCl was constructed in three different mediums such as pH1.2, 7.4, and 6.8 respectively by measuring the absorbance of Tramadol HCl solution of concentrations ranging from 2-10ug/ml. The absorbance data and calibration curve is shown in tables 19-21 and figures 9-11. The calibration curve obtained from all the three different medium showed regression value of about 0.994-0.998. The higher regression value obtained indicates good linearity between concentration and the measured absorbance for Tramadol HCl

Table 19: Calibration curve data of Tramadol hydrochloride in buffer pH 1.2

Concentration (µg)	Absorbance (271nm)
0	0
2	0.021
4	0.032
6	0.045
8	0.067
10	0.082

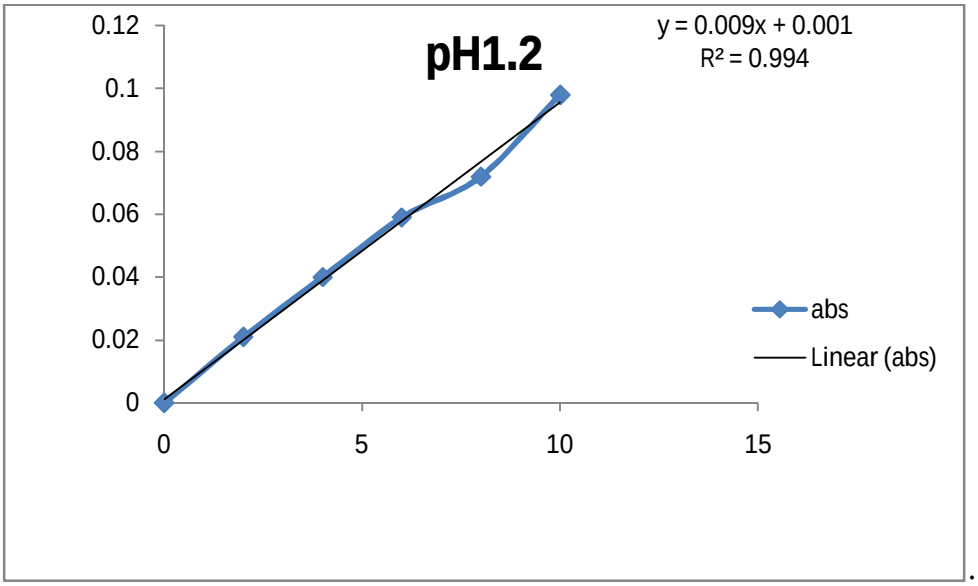


Fig 9: Standard graph for pH 1.2 acid buffer

Table 20: Calibration curve data of Tramadol hydrochloride in buffer pH 7.4

Concentration (µg)	Absorbance (271nm)
0	0
2	0.019
4	0.032
6	0.055
8	0.064
10	0.087

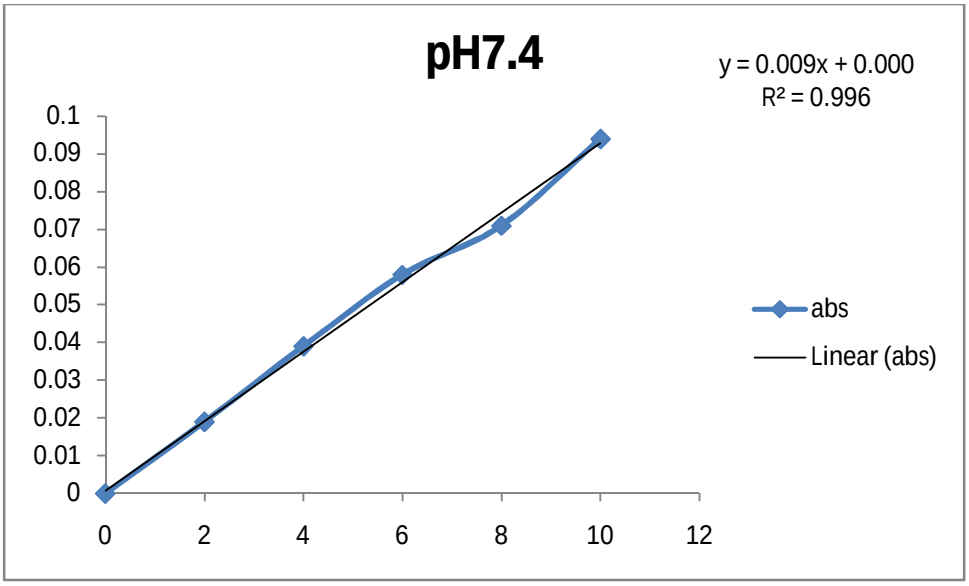


Fig 10: Standard graph for pH 7.4 phosphate buffer

Table 21: Calibration curve data of Tramadol hydrochloride in buffer pH 6.8

Concentration (µg)	Absorbance (271nm)
0	0
2	0.013
4	0.026
6	0.034
8	0.062
10	0.065

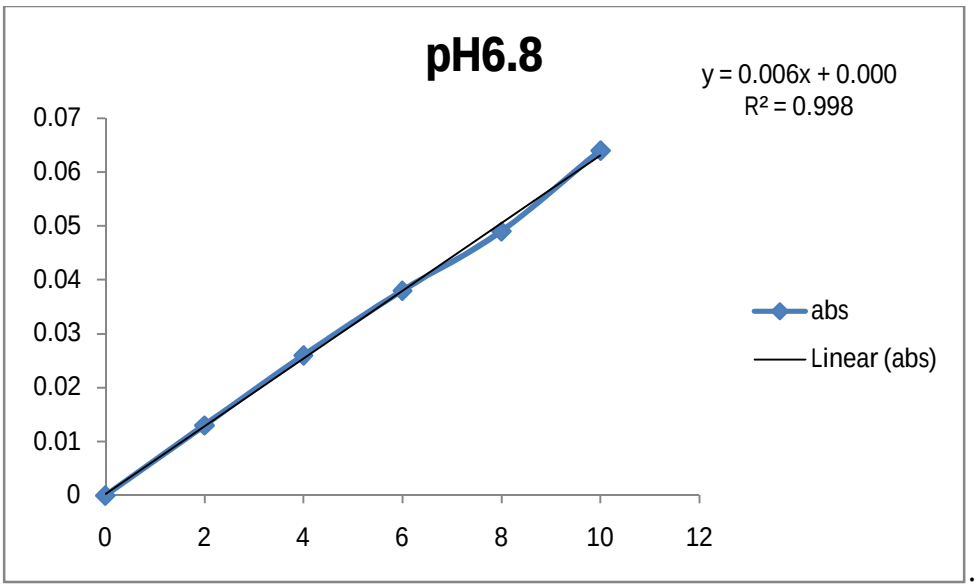


Fig 11: Standard graph for pH 6.8 phosphate buffer

6.3 Preformulation study:

Preformulation studies for the prepared powder blend were performed in order to evaluate the physicochemical properties and to analyze whether the prepared blend will be suitable for preparation of tablets using direct compression method. Various parameters evaluated are Angle of repose, Bulk density, Tapped density, Compressibility index and Hausner's ratio. The results obtained from the above studies are shown in table 22. The powder blend had an angle of repose of about 23^0 suggesting that the powder is having good flowability. The Carr's index was found to be 17.2% and Hausners ratio was found to be 0.82 these values indicates that the prepared powder blend have sufficient flowability and compressibility and hence can be processed comfortably by direct compression method.

Table no22: Result for preformulation studies:

ANGLE OF REPOSE (degree)	**BULK DENSITY (gm/cm^3)**	**TAPPED DENSITY (gm/cm^3)**	**CARR'S INDEX (%)**	**HAUSNERS RATIO**
23^0	0.66	0.54	17.2	0.82

6.4 Compression of tablets:

Core tablets of Tramadol HCl were compressed by using 16 station rotary tablet punching machine (CADMACH Ahmadabad) to a target weight of 550mg. There were no significant processing problems during the compression process and all the prepared tablets were found to be uniform in shape and size.

6.5 Coating of tablet:

The core tablets prepared were then coated with three different polymers such as Eudragit E100, HPMC and Eudragit S 100. The immediate coat on the core tablet was done with Eudragit E100. Initially Acetone was used as solvent for Eudragit E100 and was used for coating of core tablet. During the coating process the polymer solution with acetone as solvent could not produce a proper coat on the tablets. Acetone being highly volatile and the higher temperature inside the coating pan lead to rapid evaporation of solvent before it reaches the tablet bed and hence the tablet got rough appearance on the surface and the coating was not efficient. In order to avoid this problem the solvent was changed to Isopropyl Alcohol (IPA). The IPA solution produced a uniform and smooth coating over the core tablets. Coating was continued till there was a net weight increase of about 3% of the average weight for first batch, 6% for the second and 9% for the third formulation. The tablet coated with Eudragit E100 was subjected to barrier coating using HPMC as the polymer. A barrier coating was done until a net weight increase of about 5% was achieved for all the formulations.

A third layer coating was done to the barrier coated tablet using Eudragit S100 to make them resistant to gastric fluid. IPA was used as solvent for Eudragit S100 and polyethylene glycol and talc was added as a plasticizer. The tablets were coated in three batches to a target weight increase of about 3%, 6%, and 9% respectively. The coated tablets were found to be smooth without any defects and the coating was uniform throughout the batches.

6.6 Evaluation of tablets:

The core tablets and coated tablets were evaluated for various evaluation tests such as hardness, thickness, friability, weight variation, drug content etc. The results obtained from the above studies were given in table 23 and 24. The hardness of core tablet ranged from 6.16 to 6.66kg/cm^3. These values indicate that the prepared tablet have sufficient mechanical strength to withstand any sort of pressure during handling and storage. The hardness after coating was found to be slightly increased with values ranging from 6.63 to 7.3. This increase in hardness of the tablet after coating suggests that the polymers have given more strength to the tablet.

The friability test done on the core tablet gave a percentage weight loss of about 0.059 to 0.426 which is well within the prescribed limit of 1% as per the IP. Interestingly friability values for the coated tablets were almost 0% for all the formulations except F-1 which shows value of 0.0138% weight loss. These values pronounced that the coating of the polymer was complete and uniform throughout all the formulation and they have given tremendous strength to these tablets such that they can withstand any sort of mechanical and physical shocks either during handling and storage or during transportation.

Table no 23: Results for evaluation of tablet before and after coating:

FORMULA CODE	HARDNESS BEFORE COATING Kg/cm^2	HARDNESS AFTER COATING Kg/cm^2	FRIABILITY BEFORE COATING (%)	FRIABILITY AFTER COATING (%)
F-1	6.433 ±0.0577	6.63 ±0.057	0.1815	0.0138
F- 2	6.5 ±0.1732	6.63 ±0.115	0.1054	0
F-3	6.66 ±0.115	6.87 ±0.057	0.3555	0
F-4	6.63 ±0.057	6.733 ±0.115	0.2289	0
F-5	6.66 ±0.208	6.86 ±0.208	0.1062	0
F-6	6.533 ±0.635	6.60 ±0.1	0.0761	0
F-7	6.34 ±0.152	6.59 ±0.1	0.0599	0
F-8	6.16 ±0.404	6.67 ±0.057	0.4269	0
F-9	6.34 ±0.46	7.0 ±0.2	0.0760	0

Drug content in the prepared formulations were analyzed by performing the assay as per the procedure given in methodology section. The assay value ranged from 98.05 to 101.73% for the core tablet and the values did not show any significant changes for the coated tablets. These results clearly demonstrate that the coating procedure of the polymers used for coating does not have any significant impact on the drug content and also there was no interaction between the drug, polymer and the solvent used.

Table no 24: Results for evaluation of tablet before and after coating:

FORMULA CODE	THICKNESS(mm)	DRUG CONTENT (%) BEFORE COATING	DRUG CONTENT (%) AFTER COATING
F-1	5.133 ±0.1154	99.61	99.59
F-2	5.133 ±0.058	98.05	98.00
F-3	5.166 ±0.1527	99.21	99.21
F-4	5.233 ±0.0578	98.95	98.93
F-5	5.233 ±0.1527	99.85	99.85
F-6	5.066 ±0.1154	98.67	98.67
F-7	5.233 ±0.1527	100.59	100.28
F-8	5.233 ±0.1527	101.73	101.67
F-9	5.233 ±0.0577	99.98	100.04

Weight variation test for the core tablet and coated tablet after each stage of coating was performed as per pharmacopoeial procedure. Since the coating target was fixed based on net increase in average weight of the tablet it was necessary to check the weight variation after each stage of coating with the three different polymers. Average weight obtained for the core tablet and for tablet after each coating is within the prescribed limits of ±5% and hence all the formulations passes the weight variation test. The average weight increase after each stage of coating can be clearly seen in the table 25 this increase in weight clearly indicates that coating has been done efficiently and the required net increase in tablet weight was achieved.

Table no 25: Results for weight gain after different layers of coating in all batches:

FORMULATION CODE	WEIGHT OF TABLET BEFORE COATING (mg)	WEIGHT OF TABLET AFTER FIRST COATING (mg)	WEIGHT OF TABLET AFTER SECONDCOATING (mg)	WEIGHT OF TABLET AFTER THIRD COATING (mg)
F-1	553 ±0.0087	564 ±.00309	588 ±0.0016	610 ±0.0017
F-2	550 ±0.0106	562 ±0.0028	592 ±0.0029	626 ±0.0063
F-3	549 ±0.0131	562 ±0.0021	591 ±0.0045	647 ±0.0011
F-4	544 ±0.0082	579 ±0.0028	611 ±0.0013	629 ±0.0846
F-5	554 ±0.0100	580 ±0.0014	611 ±0.0011	646 ±0.0027
F-6	549 ±0.0121	581 ±0.0015	610 ±0.0016	663 ±0.0025
F-7	549 ±0.0121	594 ±0.0036	627 ±0.0034	646 ±0.0031
F-8	547 ±0.0155	596 ±0.0037	626 ±0.0056	665 ±0.0021
F-9	553 ±0.0175	593 ±0.0038	628 ±0.0039	683 ±0.0038

6.7 Compatibility studies:

IR spectra matching approach was used for detection of any possible chemical interaction between drug and polymer. Pure drug, uncoated tablet and coated tablets were taken in powder form and separately mixed with three suitable quantities of potassium bromide. About 100mg of mixture was compressed to form a transparent pellet using a hydraulic press at 6tons pressure. It was scanned from 4000 to 400 cm-1 in FTIR spectrometer. The IR spectrum of the physical mixture was compared with those of pure drug and polymers and matching was done to detect any appearance or disappearance of peaks. The IR spectrums of the tablets in the range of 4000 cm-1 to 400 cm-1 were taken by preparing dispersion in dry potassium bromide under the same operational conditions mentioned above. The spectrum obtained is shown in Figs.12, 13 and 14

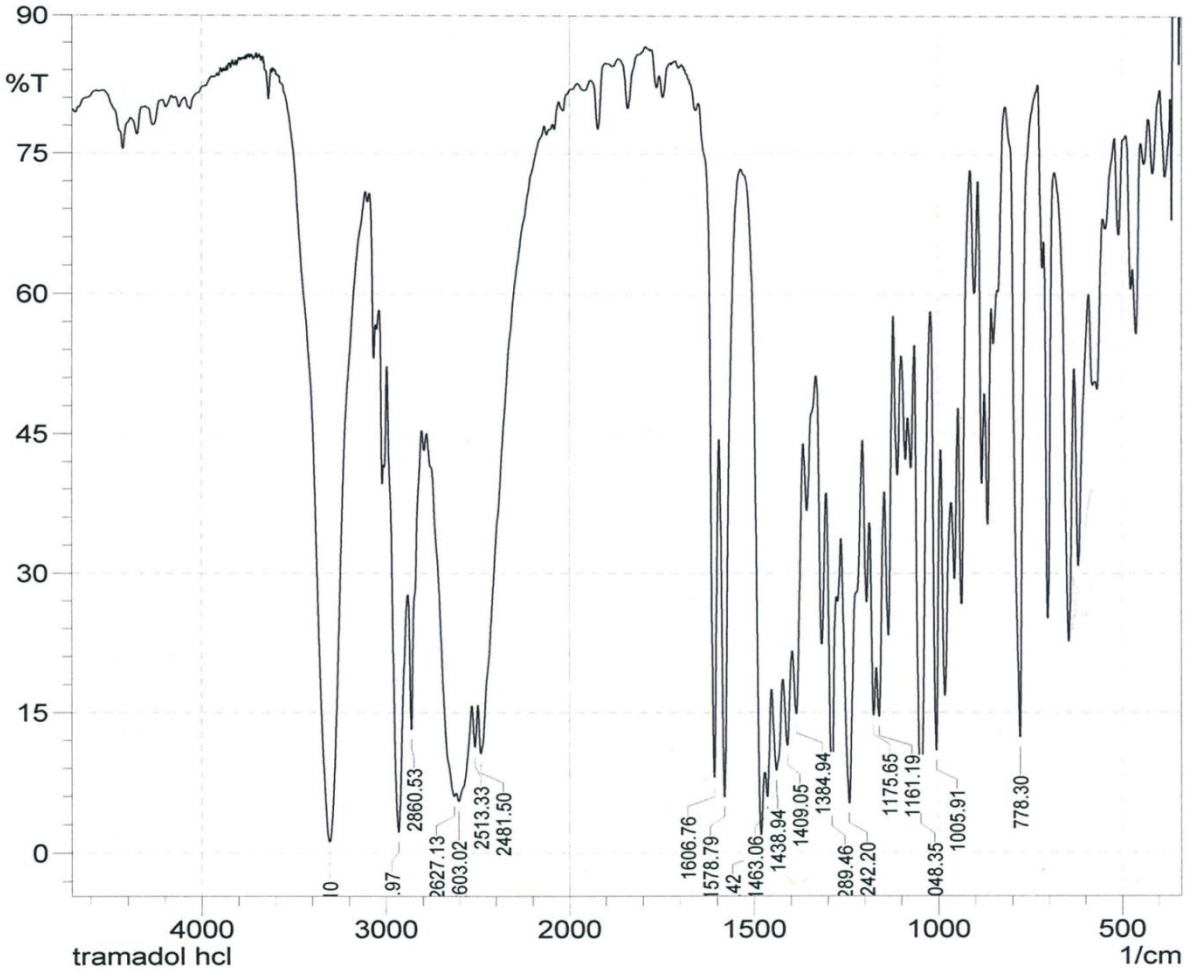


Fig 12: IR Spectra of Tramadol HCl

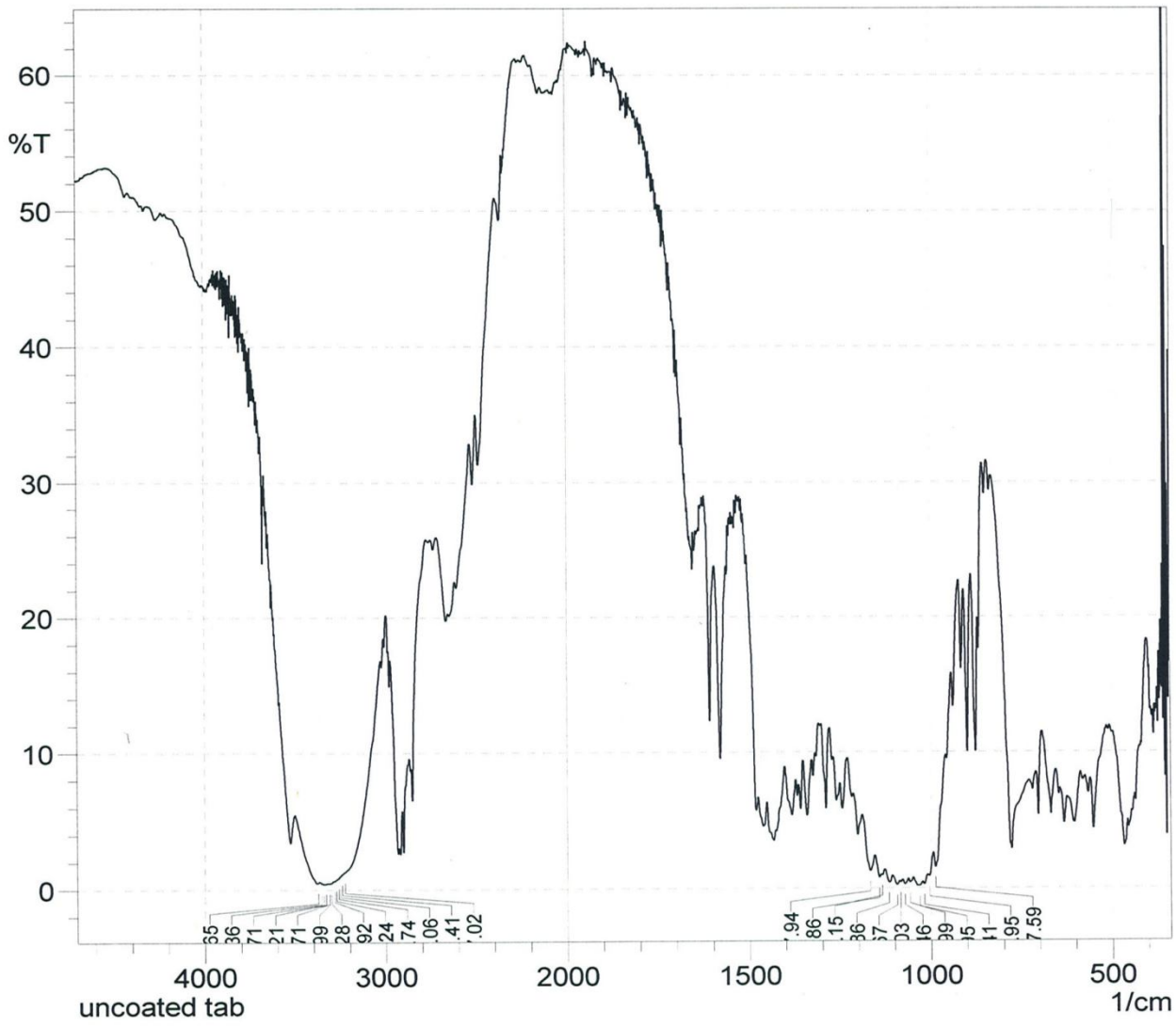


Fig 13: IR Spectra of Uncoated tablet

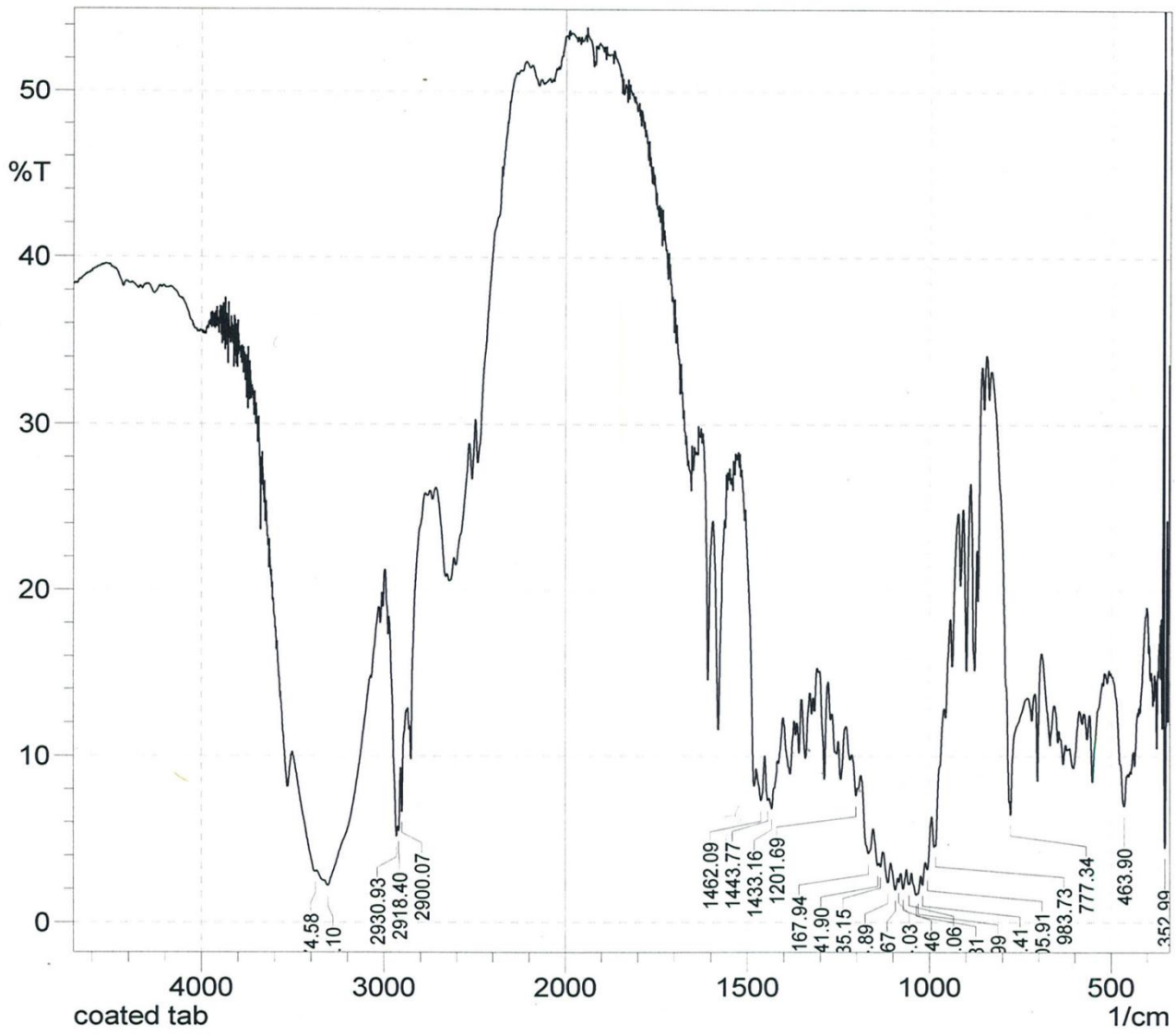


Fig 14: IR Spectra of Coated tablet

Table 26: Characteristic peaks of Tramadol HCl

SERIAL NUMBER	WAVENUMBER	SPECIFICATION
1	1048cm^{-1}	C-O-C stretch of Aryl alkyl Ether- Symmetric
2	1242cm^{-1}	C-O-C stretch of Aryl alkyl Ether- Asymmetric
3	1384cm^{-1}	C-N Stretching
4	2929cm^{-1} 2860 cm^{-1}	CH3-CH Stretching
5	3372cm^{-1}	OH Stretching Vibration

The data given in a table 26 shows the characteristic peaks detected in the IR spectrum of pure Tramadol HCl tablets. The spectra of the coated and uncoated tablets were compared with the spectra of pure drug and the presence or absence of the characteristic peaks was observed. It was found that the spectra of the tablets showed all the characteristic peaks observed in the pure sample suggesting that the drug is intact and no compatibility problems were found between the drug, polymer and other excipents.

6.8 *In vitro* drug release studies:

An ideal colon targeted drug delivery system should release the drug only in colon area to get maximum benefits with regard to drug absorption and oral bioavailability. Hence to analyze the drug release pattern of the prepared tablets, *in vitro* drug release studies were performed at three stages.

In first stage 900ml simulated gastric fluid of pH 1.2 was used as dissolution medium for two hours for mimicking the transit time in stomach.

In the second stage dissolution was continued for further three hours mimicking small intestinal transit time in 900ml simulated intestinal fluid of pH7.4.

The studies were continued for further 19 hours using 900ml simulated colonic fluid pH 6.8 mimicking large intestinal transit time. Thus dissolution studies were performed for a total of 24 hours and the result obtained were given in table 27.

The drug release data in pH 1.2 buffer showed almost nil release except in F-1 which showed a negligible amount of about 3% in the first two hours. The obtained data from second stage of the released studies in pH 7.4 gave cumulative percentage release ranging from 6.22 to 51.07% after 3 hours. The formulation F-1, F-4 and F-7 which contains 3%, 6% and 9% respectively of Eudragit S100 and 3% of Eudragit E100 gave a release of 51.07%, 39.1% and 13% respectively after 5 hours in simulated intestinal fluid. From the values we can observe that as the level of Eudragit S100 was increased the percentage release of drug was found to be decreased, Similar pattern of release was also found in F-2, F-5 and F-8 formulation coated which contains constant 6% of Eudragit E100 with 3%, 6% and 9% Eudragit S100 gave values of 47.58, 26.79 and 7.13% respectively. Formulation F-3, F-6 and F-9 having 9% Eudragit E100 coating solution with 3, 6 and 9% Eudragit S100 coating gave a cumulative release of 39%, 21.10% and 6.22% at 5h in simulated intestinal fluid and is depicted in fig 15,16 and17.

Table no 27: Results for *in vitro* release studies (% cumulative release):

Time	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
0.5	0	0	0	0	0	0	0	0	0
1	1.11	0	0	0	0	0	0	0	0
1.5	2.06	0	0	0	0	0	0	0	0
2	3	0	0	0	0	0	0	0	0
2.5	3.22	1.71	1.01	0.99	0.50	0.50	0.23	0.00	0.00
3	9.50	7.83	5.59	3.24	2.30	2.01	2.00	2.98	1,88
3.5	16.07	13.33	11.98	7.20	5.11	4.99	3.77	3.73	2.07
4	25.73	24.00	19.11	16.31	14.16	12.42	7.15	5.87	3.42
4.5	39.98	31.31	24.43	27.43	21.27	16.55	10.39	8.91	5.01
5	51.07	47.58	39.10	39.10	26.79	21.10	13.00	7.13	6.22
5.5	60.01	52.72	40.09	41.01	27.00	22.22	15.22	12.10	7.00
6	68.11	55.60	48.72	44.70	31.98	24.89	18.19	16.01	9.10
6.5	73.03	59.58	51.91	47.70	37.72	27.01	23.74	21.11	12.00
7	75.51	63.25	57.63	51.03	44.00	33.73	27.15	24.00	16.03
7.5	81.22	71.44	62.99	56.65	50.60	39.00	33.17	26.72	19.23
8	89.13	83.36	75.77	59.99	66.60	45.12	38.01	30.33	27.72
8.5	95.43	89.32	79.98	65.51	68.36	51.21	45.73	35.44	33.98
9	97.71	93.16	84.21	73.57	70.04	55.75	52.41	41.99	40.78
9.5	98.00	95.10	89.95	77.42	72.33	58.27	57.42	45.09	50.76
10	98.09	95.11	90.41	81.21	73.59	64.88	62.15	47.16	55.76
11			93.33	83.32	75,07	67.22	64.79	51.40	59.98
12			96.01	88.78	77.04	71.51	68.01	55.41	59.43
13			97.39	90.01	81.89	75.24	71.71	61.73	61.64
14			98.02	92.23	85.05	81.00	78.32	69.37	64.07
16			98.09	95.14	90.51	85.44	80.62	74.33	68.38
18				98.50	98.07	88.73	97.16	89.77	73.33
20				98.95	98.20	97.16	98.02	92.89	76.77
24								99.04	83.98

On careful observation of above data it can be understood that the drug release tend to decrease with increase in the polymer content of both E100 and S100.

The third stage of dissolution study was performed for a further period of 19 hrs using simulated colonic fluid pH 6.8. The dissolution profile showing the effect of Eudragit E100 is depicted in fig 18, 19 and 20. It was interesting to note that the pattern of release follows a similar way in simulated colonic fluid also. The drug release was almost complete with F-1 and F-2 within 10 hrs of the study where as the formulation F-3 shows a complete release at 16 hrs followed by F-4, F-5, F-6 and F-7 which gave complete release of the drug within 20 hrs of the study. Formulation F-8 with 9% Eudragit S100and 6% E100 gave a near 100% release after 24hrs which seems to be the best among 9 formulations. Formulation F-9 gave a percentage cumulative release of about 83% at the end of 24hrs which suggest that the formulation could not release its entire content within the desired time limit of 24hrs.

These data suggest that the formulation F-8 can be chosen as the best among 9 formulations prepared as it produced maximum release in colonic fluids (>90%) and also gave complete release in 24hrs.

Effect of Eudragit S100 on the drug release:

The dissolution profile of formulations (F1, F4 & F7) containing 3, 6 & 9% coating level of S100 and 3% of E100 is shown in Fig.15. Eudragit S100 is an enteric polymer which will not dissolve in simulated gastric fluid and hence the prepared formulations did not show any drug release in the first two hours when the dissolution was performed in pH 1.2 buffer. In the next stage, when the studies were continued in pH 7.4 buffer, the polymer coating of S100 gets dissolved and exposes the barrier coating layer of HPMC. HPMC being hydrophilic in nature tends to swell and disintegrate and thus exposes the Eudragit E100 layer. Although Eudragit S100 is completely soluble in pH 7.4, the formulations with increasing S100 content exhibited a decrease in the amount of drug released during the second stage of the releases studies. This can be explained by the fact that formulations F-1, F-4 and F-7 containing 3% Eudragit E100 and 3, 6 & 9 % Eudragit S100 gave a cumulative release of 51.07, 39 & 13% respectively. These data

suggests that when the coating level of Eudragit S100 was increased, the time taken for the complete dissolution of the coating also gets increased and hence the cumulative release of the drug was reduced. The dissolution profiles of formulations with 6% & 9% Eudragit E100 were shown in fig.16&17. These profiles also follow a similar pattern of release producing a decrease in the percentage release of drug with increase in the polymer content.

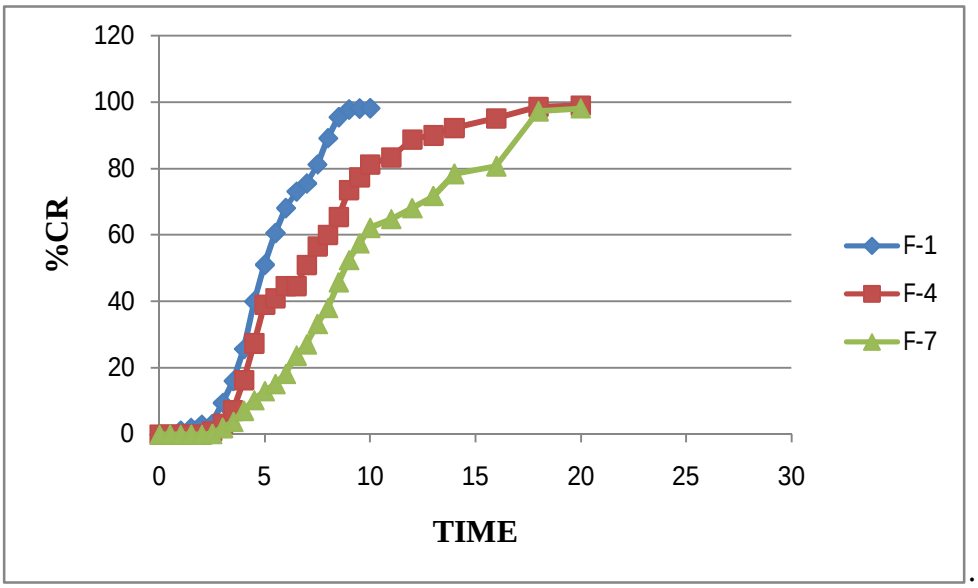


Fig 15: Dissolution Profile of 3, 6, and 9% Eudragit S100 and 3% Eudragit E100

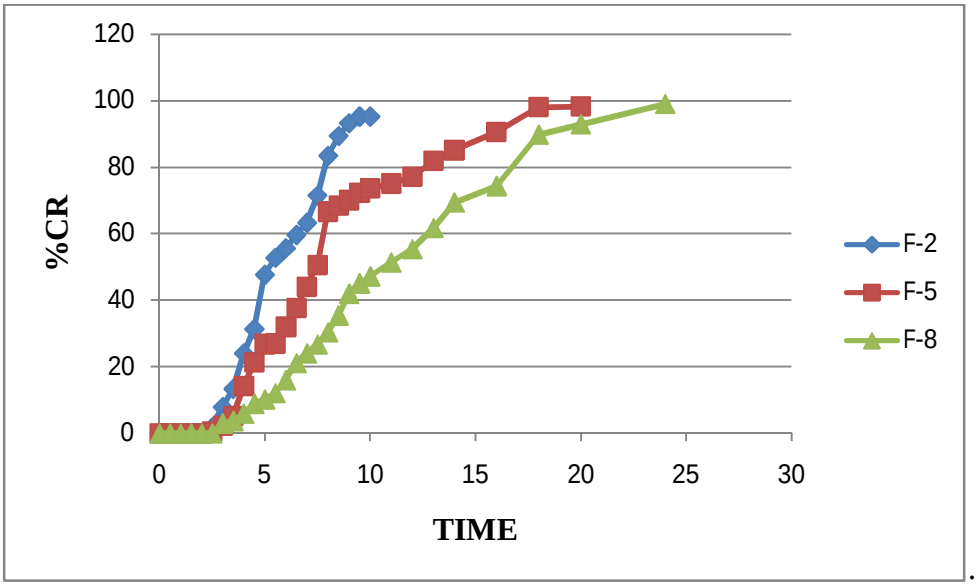


Fig 16: Dissolution Profile of 3, 6, and 9% Eudragit S100 and 6% Eudragit E100

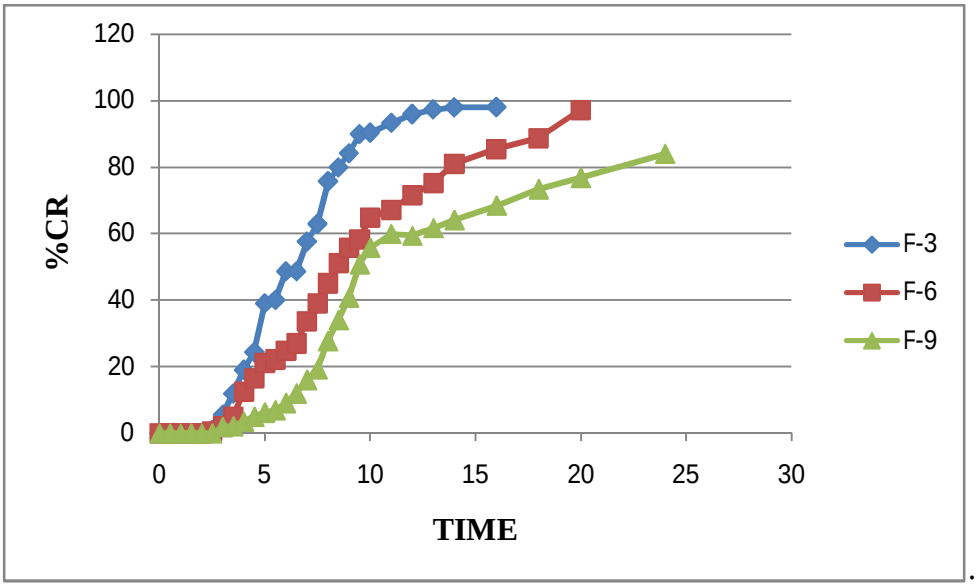


Fig 17: Dissolution Profile of 3, 6, and 9% Eudragit S100 and 9% Eudragit E100

Effect of Eudragit E100 on drug release

Eudragit E100 is an acid soluble polymer, which is insoluble at pH 7.4 and 6.8. But it becomes more permeable at a pH of above 5.5. In the second stage of the dissolution studies where pH 7.4 buffer was used, the layer of S100 coating and barrier layer of HPMC gets dissolved completely exposing the E100 layer above the core tablet. Since the polymer is permeable above pH 5.5, it allows for the drug permeation and hence drug is released slowly from the core tablet. The dissolution profiles of formulations with constant amount of S100 coating and varying amount of E100 coating were shown in Fig.18, 19 & 20. The percentage release of the drug after 3 hours in pH 7.4 buffer gave values ranging from 6.22% for F-9 to 51.07% for F-1 formulations.

Careful observation of the dissolution data reveals the fact that the drug release was inversely proportional to the polymer content in the coating layer. This can be explained with the following example. The formulations with 3% S100 coating and 3, 6 & 9% E100 coatings gave a cumulative release of 51.07, 47.58 & 39.10% after 5h of the study. The same formulations released about 98.09%, and 95.11% in 10h, and 98.09% in 16h respectively in colonic pH. The

formulation with 3% E100 coating released the entire contents within 10h, whereas formulations with 6 & 9% coating of E100 produced a prolonged release for 16h. Similarly, formulations F4, F5 & F6 with a constant 6% S100 coating and 3, 6 & 9 % coating of E100 polymer gave a cumulative release of about 98.95%, 98.2% and 97.16% respectively, in 20h.

From the above data, it is evident that the polymer content plays a significant role in altering the drug release characteristics of the tablets.

On basis of the *in vitro* release characteristics of the prepared formulations, formulations F8 & F9 containing 9% Eudragit S100 coating along with 6 & 9% Eudragit E100 coating respectively were selected as the best among the nine formulations. These two formulations gave a release of more than 90% in the colonic pH and a meager 7.13 & 6.22 % in the intestinal pH making them most suitable for colon targeting. Among these two, F8 with 9% S100 coating and 6% E100 coating was selected as the best one, based on the reason that it produced maximum release in the colonic pH and also gave almost 100% release in 24h whereas formulation F9 could produce only 83.98% release at the end of 24h.

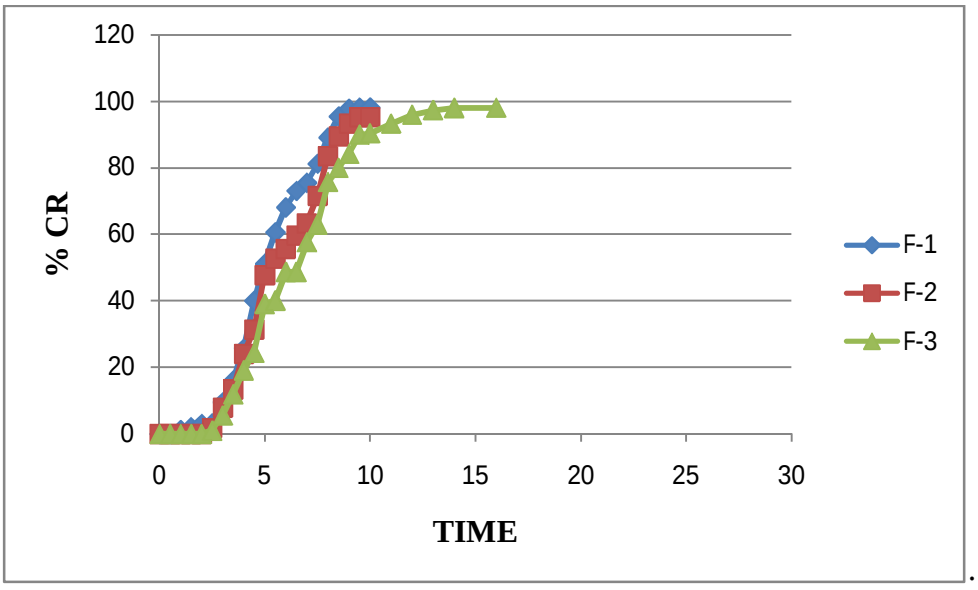


Fig 18: Dissolution Profile of Formulation F-1, F-2and F-3

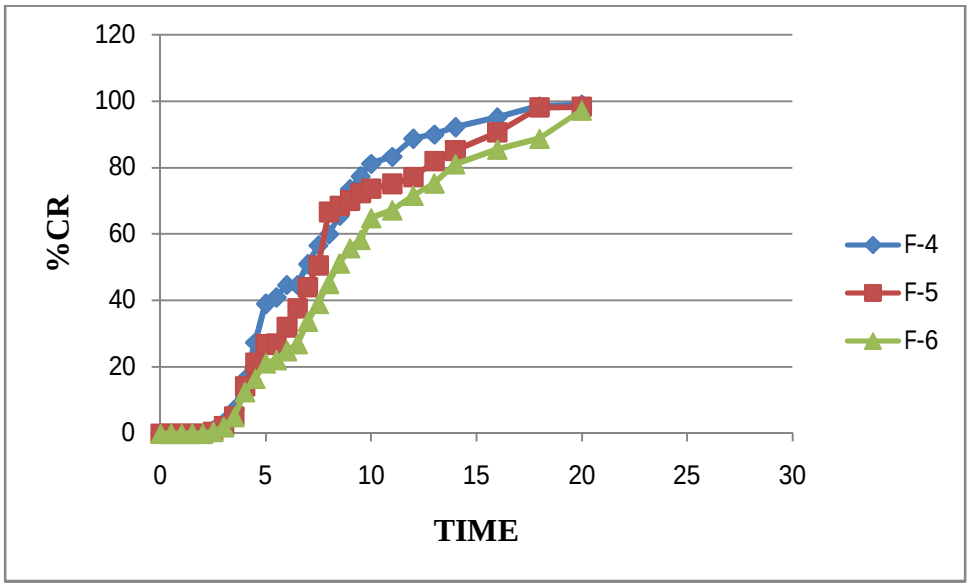


Fig 19: Dissolution Profile of Formulation F-4, F-5and F-6

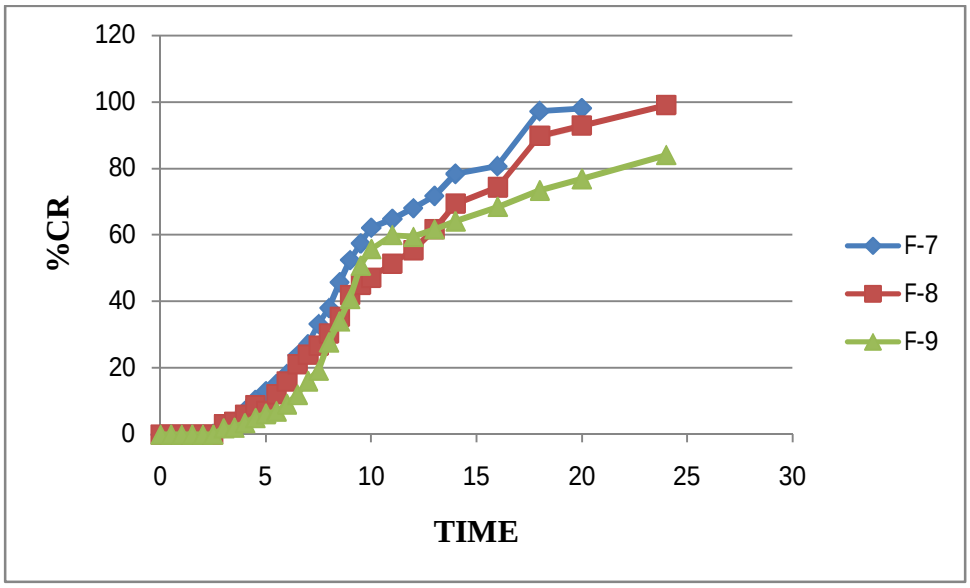


Fig 20: Dissolution Profile of Formulation F-7, F-8and F-9

6.9 Kinetic analysis

The *in vitro* drug release data were subjected to kinetic analysis by plotting various kinetic equations like zero order, first order, Higuchi, and Hixon crowells plot. They were also subjected for peppas plot in order to find out the mechanism of release from the prepared tablets . The kinetic analysis data of all the formulations were shown in Table no 28.

The kinetic model that best fits with the release data of formulation was evaluated by the correlation coiefficient (R^2) values. According to the values obtained it was found that F-1, F-2, F-6, F-7 and F-8 showed a higher linearity with zero order plots with R^2 values ranging from 0.953 to 0.969 indicating controlled release of drug from the prepared formulation . Formulation F-3, F-4, F-5 and F-9 gave a best fit for first order equation describing drug release rate with concentration of drug.

Mechanism of drug release:

Mechanism of drug release data can be assist by plotting the drug release data with Koresmeyer-peppas equation. The release exponent (n) value indicates the mechanism of drug transport from the matrix system. When the n value is equal to 0.5 indicates Fickian diffusion or anomalous, 0.45-0.89 indicates non-fickian transport more than 0.89 indicates Super case II transport.

The slope values obtained from the formulation ranged from 1.629 to 2.071 revealed the fact that drug release follows super case II transport diffussion posibly due to polymer chain errosion and swelling.

Table 28: Release kinetics parameter

Formulation	Zero order	First order	Higuchi	Koresmeyer-peppas		Hixon crowell
	R^2	R^2	R^2	R^2	n	R^2
F1	0.956	0.841	0.845	0.939	1.986	0.910
F2	0.960	0.844	0.830	0.890	2.071	0.895
F3	0.915	0.925	0.876	0.883	1.893	0.783
F4	0.891	0.949	0.899	0.871	1.779	0.709
F5	0.906	0.914	0.889	0.856	1.795	0.742
F6	0.953	0.908	0.893	0.870	1.748	0.912
F7	0.956	0.829	0.862	0.847	1.722	0.832
F8	0.969	0.825	0.876	0.919	1.629	0.804
F9	0.899	0.940	0.831	0.882	1.633	0.800

6.10 Stability study

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions. Formulation F8 which was chosen as the best one was loaded at accelerated condition at $40^0C \pm 2^0C$ and 75% RH±5%RH in a stability chamber. Samples were withdrawn at 30, and 60, days and analyzed suitably for the drug content and drug release and the results are shown in Table 29.

Table 29: Stability study of Tramadol hcl Tablets formulation 8:

Duration	Drug content (%)	Percentage cumulative drug release		
		pH1.2	pH7.4	pH6.8
Initial	99	0	7.13	99.04
30 Days	98	0	6.8	97.78
60 Days	99	0	8.1	98.45

The results of stability studies indicate no significant changes in the drug content and drug release characteristics which provide evidence for better stability of the prepared formulations in accelerated stability conditions. It also suggests that the ingredients used in the formulations were compatible with each other and no interactions were found between them.

Summary

9. SUMMARY

The present study was aimed to prepare colon targeted tablets containing Tramadol HCl as active pharmaceutical agent and Eudragit E100 and Eudragit S100 as pH dependent polymers and to study the effect of these polymers on drug release rate. Since the oral bioavailability of Tramadol HCl is poor due to first pass metabolism, colon targeted tablets can be used as a promising formulation to by-pass the first past effect and thereby improve bioavailability.

Formulation

Colon targeted tablets of Tramadol HCl was formulated by using a different pH dependent polymers such as Eudragit E100 and Eudragit S100. Different layers of polymers has been coated on the core tablet by using HPMC as a barrier layer coat to avoid all possible interaction between Eudragit E100 and S100 since they are oppositely charged and different plasticizers were also used to give a proper coating and strength to the tablet. The coating of pH dependent polymers was done at three coating levels to give a net weight increase of about 3, 6 and 9% respectively. The barrier layer coat with HPMC was done to a constant weight increase of about 5%. Thus the core tablets have three layers coating comprising Eudragit S100 as the outer coat, HPMC as the middle layer barrier coat and Eudragit E100 as the innermost coat.

Evaluation

Evaluation studies were performed which includes all the preformulation and post formulation studies. The core and coated tablets evaluation studies includes hardness, friability, weight variation, drug content, thickness test and weight gain after each successive coating.

In vitro drug release study was done in USP type II Paddle apparatus in different pH media such as pH1.2 for two hours, pH7.4 for three hours and pH6.8 for nineteen hours to mimic the intestinal pH and transit time. Sampling was done in prescribed time limit and analyzed for drug content using UV spectrophotometer for calculating the drug release.

IR studies were done to confirm the compatibility of the pure drug and polymers. Stability studies were also performed for the best formulation for a period of 60days by storing the product in accelerated temperature and humidity conditions in a stability chamber and observed for any changes in appearance and drug release rate from the formulation.

Results and Discussion

The preformulation studies performed on the powder blend showed satisfactory results with respect to the flow property and compressibility. The values obtained as angle of repose and compressibility index suggested that the prepared blend is well suitable to be compressed as tablets using direct compression method. The tablets were compressed by using 16 station rotary compression machine and the prepared tablets were found to be uniform in shape and size, with smooth surface and texture. There were no processing problems encountered during the compression process. Coating of the core tablets were done by using three polymers in the following order: Eudragit E100 – HPMC – Eudragit S100. The innermost layer was the acid soluble E100 polymer, the middle layer was HPMC which acts as the barrier coat and the outermost layer was S100 which is a gastro resistant polymer. Coating was done to three levels of weight gain i.e 3, 6 & 9% for E100 and S100, and weight gain was kept at a constant 5% for HPMC coating.

The coated tablets were evaluated for weight gain after each coating levels to ensure the efficiency of the coating process. Further the tablets were also subjected to various evaluation parameters prescribed for coated tablets. The post compression evaluation of the coated tablets were found be within the prescribed limits of Indian Pharmacopeia with respect to weight variation, friability, content uniformity etc.

The drug release studies were performed in three different media mimicking the various areas of the GIT viz. stomach, intestine and colon. Studies were done for a period of 24h which includes 2h in gastric medium, followed by 3h in intestinal medium and remaining 19h in colonic medium. All the nine formulations did not produce any drug release in the first 2h in gastric medium except F-1 which gave a release of about 3% indicating that Eudragit S100 coating was done effectively, which protected the drug in the acidic environment.

The drug release in the intestinal conditions was found to be in the range of 6.22% to 51.07% in the next three hours. The data suggest that as the polymer concentration was increased the drug release was found to be decreased. Formulation F-1 with 3% levels of E100 and S100 gave a release of about 51.07% whereas F-9 with 9% E100 and S100 gave only 6.22% which supports

the above statement. Similarly in the colonic pH conditions also, the release was found to be following the same pattern as that of in the intestinal conditions. Careful analysis of the drug release data revealed the fact that formulations F8 and F9 gave more than 90% release in the colonic pH conditions with less than 10% release in the gastric and intestinal conditions. Hence these two formulations were seems to be more appropriate for delivering maximum drug to the colon. Out of these two, formulation F8 with 9% S100 and 6% E100 coatings was selected as the best formulation based on the fact that it gave 93% release in the colonic condition and also gave 100% release in 24h, whereas formulation F9 with 9% Eudragit E100 and Eudragit S100 coating although gave more than 90% release in colon, could only produce 83% release in 24h.

Kinetic analysis of the release data obtained from the formulations suggested that most of the formulations (F1, F2, F6, F7 & F8) showed high linearity for zero order equation followed by first order kinetics (F3, F4, F5 &F9). The mechanism of release according to Peppas equation was found to be super case II transport, as the 'n' values were found to be more than 1, suggesting diffusion through polymer chain erosion and swelling.

Stability studies indicated that the prepared formulations were stable at accelerated temperature and humidity conditions, showing no signs of physical or chemical degradation.

Thus in this study, colon targeted delivery system for Tramadol HCl was formulated successfully and the results obtained from the work was encouraging. The optimized formula will be taken up for further studies for pilot scale up batch.


Conclusions?

10. CONCLUSION

Delivery of drugs to the systemic circulation without intermittent loss due to improper absorption, degradation in gastric conditions, first pass metabolism etc, is a challenging task for the formulation scientists. Although many approaches have been attempted for providing maximum bioavailability, colonic drug delivery has emerged as one of the promising method for improving bioavailability, targeting the drug for local effects in the colon, bypassing the first pass effect etc.

In this study, we have made an attempt to develop an oral system for colon targeting using Tramadol HCl as the model drug. Nine formulations were developed by coating pH dependent polymers such as Eudragit E100 and S100 in different coating levels to delay the drug release in GIT. The observed results from the study have demonstrated the efficiency of these pH dependent polymers in delivering the drugs in the colonic region. It was also evident that when the coating levels of these polymers were increased, the drug release tends to slow down and thus enables the formulation to release the contents in colonic pH. Formulation F8 with 9% coating level of Eudragit S100 and 6% coating level of Eudragit E100 along with 5% HPMC barrier coating was found to be the optimized one as it showed more than 90% release in the colonic condition, with negligible release in the intestinal pH and it also gave a complete release in 24h.

It can be concluded that, the combination of Eudragit E100 and S100 can be used effectively for colon targeting of drugs. Further animal studies for the *in vivo* results are necessary to claim complete success from the study.

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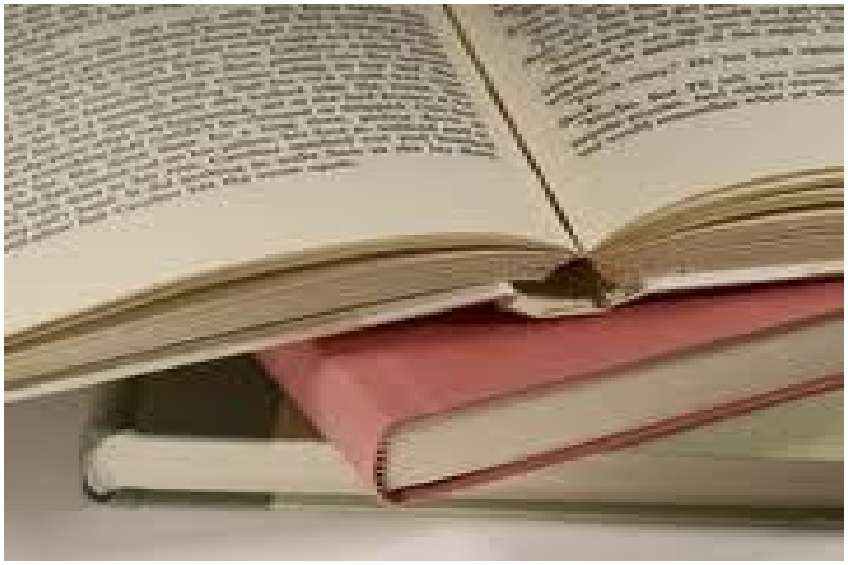

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